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STUDIES OF THE ASCIGEROUS STAGE OF VENTURIA INAEQUALIS (CKE.) WINT. IN RELATION TO CERTAIN FACTORS OF THE ENVIRONMENT[1]

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The recent work of Keitt and Jones (8) has shown that, under conditions where ascospores are the only important source of inoculum for primary infection of the apple by *Venturia inaequalis* (Cke.) Wint., the presence of an abundant and timely ascosporic inoculum is one of the chief requisites for the development of epidemic outbreaks of the scab disease. Since the ascospores comprise the only important primary inoculum known in most apple-growing sections, any practical means of limiting their timely production and discharge would be of great potential value in the economic control of the disease. Inasmuch as the work hitherto done on apple scab has been concerned chiefly with the parasitic phase of the pathogen and with the development and control of the disease, it seemed desirable to give special attention in the present work to the saprophytic phase of the fungus in relation to some of the more important factors which influence its development. It is hoped that a more adequate understanding of the development of the ascigerous stage of the parasite may be of value in furthering the understanding of the epidemiology and control of the disease.

For a more general discussion of apple scab problems and for citations to the literature of the disease, reference may be made to the paper of Keitt and Jones (8) and the works cited therein.

RELATION OF LEAF INFECTION TO THE PRODUCTION OF THE ASCIGEROUS STAGE

In studying the quantitative aspects of the production of ascosporic inoculum, one of the first points for consideration is the relation of leaf

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infection to the production of perithecia. The following questions seemed worthy of consideration: Do the type and abundance of leaf lesions influence the production of perithecia? Does the mycelium of the fungus ramify the tissues of the dead leaves extensively and produce perithecia at considerable distances from lesions? Does the fungus spread from dead leaves which have been infected by scab into those which have not been infected and there produce perithecia?

Type of Lesion

It has long been observed that two general types of scab lesion occur on the leaves of the apple. The more conspicuous is the rather definitely margined type, which occurs very generally on the upper (ventral) surface of the leaf. Less conspicuous is the indefinitely margined type, which is found very commonly on the lower (dorsal) surface. There are gradations between these two types and neither is restricted to one surface of the leaf. Aderhold (1), Clinton (2), and Wiltshire (16) found that the younger leaves of the apple were more susceptible to infection by *V. inaequalis* than older leaves. Keitt and Jones (8) found that the upper (ventral) surface of the apple leaf becomes resistant more rapidly than the lower, and that the period of incubation is lengthened as resistance increases. The lesions which occur after considerable resistance has been developed, particularly those on the lower surface, are commonly diffuse and inconspicuous. The present writer has noted that, under field conditions, the majority of the lesions which appear during the latter part of the growing season are of the diffuse or indefinitely margined type.

Considerable practical importance attaches to the relation of the two types of lesions to the production of perithecia. Lawrence (11) reports that lesions which appear late in the season and produce few conidia produce perithecia in greater numbers than those which had borne conidia in large numbers. Killian (10) states that the cells at the center of the lesions die, and penetration of the inner tissues of the leaf takes place only at the periphery. The present writer has compared the two types of lesion in this regard in leaves cleared by the chloral hydrate-potassium hydroxide method (Peace, 12). Perithecia were produced commonly only beneath the periphery of the more definitely margined lesions, while they were produced in any part of the area beneath the diffuse type. It was also noted that the cells of the stromatoid layer near the center of the more definite type of lesion were frequently without protoplasmic contents, while the cells at the periphery were more or less filled with protoplasm. Comparative studies showed that the number of points of penetration of the leaf lamina by hyphae of the fungus seemed to bear a direct relation to the presence of

protoplasmic contents in the cells of the stromatoid layer adjacent to the points where the hyphae originated. There also appeared to be an inverse relationship of the number of conidiophores produced on a given area of the stromatoid layer to the presence of protoplasm in the fungal cells of that area. All parts of the stromatoid layer of the diffuse type of lesion had cells generally well filled with protoplasm. Furthermore, fewer conidiophores were usually found on this type of lesion than on the more definitely margined type. It would seem, therefore, that the diffuse type is the more important in relation to the production of perithecia, because (a) of its common occurrence in larger numbers and (b) of the possibility of production of ascocarps at any point beneath the lesion.

Spread of the Fungus in the Interior of Infected Leaves and the Relation of Number of Lesions to the Abundance of Perithecia

The extent of ramification and saprophytic development of the mycelium of the fungus in the dead leaves and the relation of abundance of lesions to the development of perithecia are of much importance to an understanding of the quantitative aspects of the development of the ascosporic inoculum. A perusal of the literature gives the impression that the perfect stage develops as the result of an extensive saprophytic growth of the fungus in the dead leaves, with the suggestion that the amount of parasitic development on the leaf is not of major importance. The observations just reported do not conform with this conception. To gain further evidence on this question, leaves which bore from one to few lesions were gathered, outlined on paper with the aim of recording the exact location and size of each lesion, and overwintered in the orchard. In the spring these leaves were brought into the laboratory and placed in a moist chamber at 16° C. In a few days, as the perithecia came to maturity, it was found that they were present in the areas covered by the lesions, but in most cases could not be found farther than one centimeter from the margins of the originally observed lesions. In a few cases the perithecia were scattered over the surfaces of the leaves, regardless of proximity to the lesions which were visible when the experiment was started. Examination of cleared fragments of these leaves showed that the fungus was present over the areas occupied by these perithecia, as very sparse stromatoid layers spreading in a very narrow, fan-like arrangement beneath the cuticle of the leaf. Very few conidiophores arose from these lesions, which explains why they were not seen when the leaves were taken from the tree.

The presence of lesions which were not macroscopically visible when the leaves were green was brought to the attention of the writer in the autumn of 1926. On September 5 a survey of the University orchard showed that

only 9 per cent of the leaves of Virginia (crab) had visible lesions. Later in the autumn, after the leaves had turned yellow, it was noted that 80 per cent of the leaves of the same trees bore numerous inconspicuous lesions. Perithecia were produced in abundance from these lesions when the leaves were overwintered. The failure to observe inconspicuous or macroscopically invisible lesions may account for the prevalent conception that the fungus ramifies the laminae of the infected leaves extensively and produces perithecia in areas comparatively remote from the lesions.

Further studies of these cleared leaves failed to show any extensive spread of hyphae of the scab fungus in the laminae. They were abundant in the area beneath the lesions but did not extend very far in a lateral direction. A few measurements showed that, in some cases, perithecia were located at a distance of one millimeter from the nearest stromatoid layer, but very few were found farther away. It is probable that temperature and moisture may influence the extent of the ramification of the hyphae. These experiments were carried out during an autumn of frequent rains and moderate temperatures, conditions which were conducive to the growth of the hyphae in the laminae of the leaves. The relations of temperature and moisture to the development of the perithecia will be discussed later.

It would seem from these observations that, under ordinary autumn conditions in Wisconsin, the abundance of lesions influences, to a marked extent, the quantity of perithecia which will be produced in the overwintering leaves. More extensive experiments are necessary, however, to determine the extent of the spread of the fungus in infected leaves during autumns of excessive moisture and moderate temperatures.

Does the Fungus Invade Non-infected Leaves on the Ground?

If the fungus is able to spread extensively by means of conidia or mycelium to non-infected leaves on the ground, the presence of a few infected leaves among the clean leaves might be sufficient to lead to the production of an abundant supply of perithecia, regardless of the severity of leaf infection during the summer. This would, obviously, complicate a system of control which was aimed at reducing the primary inoculum by preventing infection of the living leaves.

Experiments were conducted in 1925–26 and 1926–27 in which uninfected apple leaves were overwintered in contact with heavily infected leaves. In another experiment of 1926–27, Venturia-free dead leaves were sprayed with a conidial and mycelial suspension of the scab organism and kept under conditions suitable for development of the fungus, after which they were placed in the orchard for over-wintering. Repeated examinations of sub-samples of these leaves which had been brought into the laboratory

in the spring and placed in a moist condition at 16° C. for several weeks failed to show the presence of perithecia in the leaves which were free from *Venturia* at the beginning of the experiment. The leaves which bore scab lesions developed numerous mature perithecia upon being kept for a few days under these conditions as controls. The uninfected leaves were in most cases much more disintegrated than the infected leaves.

RELATION OF THE TIME OF LEAF-FALL TO THE DEVELOPMENT OF THE ASCIGEROUS STAGE

Methods

Collections of scabbed apple leaves were made at various times during the autumn and early winter of three seasons. In 1924–25 the leaves were taken from the Virginia (crab) until this variety had become defoliated, after which collections were made from an unknown variety which retained its leaves until late in the winter. In 1925–26, parallel collections were made from the Virginia (crab) and the McIntosh varieties, while in 1926–27 parallel collections were made from the Virginia and the unknown variety mentioned above. These samples were placed in the orchard in open-meshed cloth bags made from curtain material. An abundance of sod provided an ideal location for overwintering the leaves.

In the more detailed studies of 1924–25, fixations of leaves from these collections were made at intervals during the autumn, winter, and early spring, and imbedded in paraffin. Flemming's medium and chromo-acetic fixing reagents were employed. These fixations provided the material for the earlier stages of perithecial development. Of necessity, a large amount of material was sectioned for study. The Pianeze III B stain (Vaughan, 14) was found to be excellent as a means of rapidly differentiating the interior of the perithecia when details were not necessary. For the more detailed studies Flemming's triple and Haidenhain's haematoxylin stains were used. Data were gathered on the diameters and stages of development of perithecia in each collection. The fixed and stained sections were studied carefully, and measurements were made of all perithecia of *V. inaequalis*. Care was taken that measurements were made only of sections through the center of the perithecia. While diameter was found to be a fairly good criterion of development of perithecia, especially in the early stages, the data on internal change give a better idea of the stage of advancement and are therefore shown in table 1. A difficulty in using average diameter of perithecia as a criterion of their stage of advancement lies in the fact that perithecial initiation continues over a considerable period. Consequently, the later formed perithecia would tend to mask the stage of advancement of those formed earlier. In order to minimize this

TABLE 1.—*Relation of time of leaf-fall to the development of perithecia of Venturia inaequalis, Madison, Wis., 1924–25*

Date of examination	Development[a] of perithecia in scabbed apple leaves placed on the ground at stated dates:								
	Aug. 12	Aug. 26	Sept. 9	Sept. 23	Oct. 7	Oct. 31	Nov. 10	Dec. 17	Feb. 9
Oct. 16	B								
Oct. 29	C	B	B	B					
Nov. 17	C	C	B	B					
Dec. 15	C	C	C	B	A				
Jan. 20	C	C	C	B	B	B	A		
Feb. 12	D	D	C	C	B	B	B		
Mar. 18	E	E	E	D	C	C	C		
Apr. 9	F	F							O
Apr. 13	G	F							O
Apr. 19	G	G	E						
Apr. 22	H	G	E						
Apr. 26		H	F	E	D				
May 11			H	H	F		D		
May 25									
June 10					H	H	H	H	O

[a] Stages of development: A = Simple coil; B = Ascogonial; C = Filamentous; D = Pre-ascus; E = Early ascus; F = Delimited spores; G = Olivaceous spores; H = Discharge.

source of confusion, the measurements were arranged in the order of magnitude, and all but the highest 25 per cent were discarded. The average measurements referred to, therefore, are representative of the more advanced perithecia.

For the studies of the later stages of perithecial development, subsamples were taken from the collections, and the more advanced perithecia were picked from the leaves and crushed on glass slides. Tests for discharge were made by placing the moistened leaves over glass slides.

The Typical Development of Perithecia

Detailed studies of the morphology and cytology of *V. inaequalis* were not undertaken by the writer. In tracing the relation of certain factors to the development of the ascocarps, however, it was necessary to follow the grosser changes which mark their development. Such studies served (a) to avoid confusion with other leaf-invading fungi, and (b) to record definitely certain well-defined stages in ascocarp development. The valuable contributions of Killian (10) and Frey (4) were very helpful guides in this work.

The possibility of mistaking perithecia of other fungi for those of *V. inaequalis* was minimized by becoming familiar with certain definite stages in the development of perithecia of the scab fungus. Furthermore, it was found that the connection of the perithecia with the subcuticular stromatoid layer was in many cases traceable. The hyphae of *V. inaequalis* penetrated but a short distance, and the perithecia were produced immediately beneath the epidermal layer. This was especially true in leaves which had been placed on the ground rather late in the autumn.

A description of perithecial development is included here with explanations of the terms used in table 1. Killian (10), who worked with the fungus in naturally infected dead leaves, states that after leaf-fall hyphae are sent into the interior of the leaf from the stromatoid layer. He figures a hypha thus arising but does not show the details of the penetration into the interior of the leaf. The present writer has found that a very common mode of entry of the hyphae is between the walls of adjacent epidermal cells (Figs. 1–2). A single hypha may push between epidermal cells and gain entrance to the interior, or the walls may be pushed apart by the development of a group of fungal cells (Fig. 2). In many instances there was noted a wedge-shaped outgrowth of a cell of the stromatoid layer which had grown between adjacent epidermal cells and pried the walls apart. Probably such outgrowths gave rise to the groups of cells which were found frequently to have developed between the walls of adjacent epidermal cells. The peculiar habit of the fungus in forming this plectenchymatous stroma-

toid tissue is very marked in some instances. Cases have been found in dead leaves in which a layer of such tissue had been formed between the end walls of the palisade cells and the adjacent epidermal walls, while other cases have been found in which this tissue had developed in the palisade region. It very frequently happens that the development of this tissue between the walls of adjacent epidermal cells apparently ruptures them, with the result that the fungus grows into the cells (Fig. 2). It appears to take the path of least resistance. A hypha may grow between the epidermal cells as described above and come in contact with the end wall of a palisade cell, in which case it may turn and grow around the cell. In other cases the hypha may branch, one branch growing around one side of the palisade cell while the other branch grows around the opposite side. In leaves which have undergone little disintegration, the lateral spread of the fungus is much more abundant in the mesophyll than in the palisade region. On the other hand, if the leaf has undergone considerable disintegration, the hyphae ramify the palisade more freely and, at times, appear to

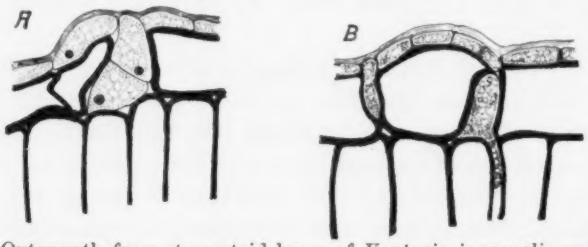

FIG. 1. A. Outgrowth from stromatoid layer of *Venturia inaequalis* pushing between the walls of adjacent epidermal cells. B. Hypha from subcuticular stromatoid layer pushing between palisade cells. Camera lucida drawings. (×625.)

pass through mesophyll or palisade cells. The intercellular habit of the fungus in the tissues of dead leaves which have undergone little disintegration suggests a partial explanation, at least, of the rather sparing lateral spread of the hyphae in the lamina as was noted earlier.

Perithecia may be produced either in the mesophyll or in the palisade regions of the dead leaf. Killian (10) figures the origin of a perithecium by the coiling of a single hypha about itself. Frey (4) describes the formation of perithecia in pure culture, both by the coiling of a single hypha about itself and by the coiling of two hyphae about each other. The young perithecium represented in Plate V, A of the present paper possessed a second hypha projecting from it at a point below the plane of sharpest focus, thus suggesting that it arose as the result of two hyphae coiling about each other. Evidence of both methods of origin has been found by the present writer in naturally infected dead leaves.

Killian (10) found that the portion of the hypha which is enclosed by the coiling of the end of the hypha goes to form the ascogonium. The ascogonial initial is at first more or less straight but soon takes on a curved aspect by segmentation and growth. The perithecial wall is formed from the segmentation and growth of the coiled portion of the hypha. He describes the formation of the trichogyne by the growth of one end of the ascogonium through the wall of the perithecium. The present writer finds that septation of the ascogonium may occur fairly early. Plate V, B represents a section through two perithecia. The perithecium at the right

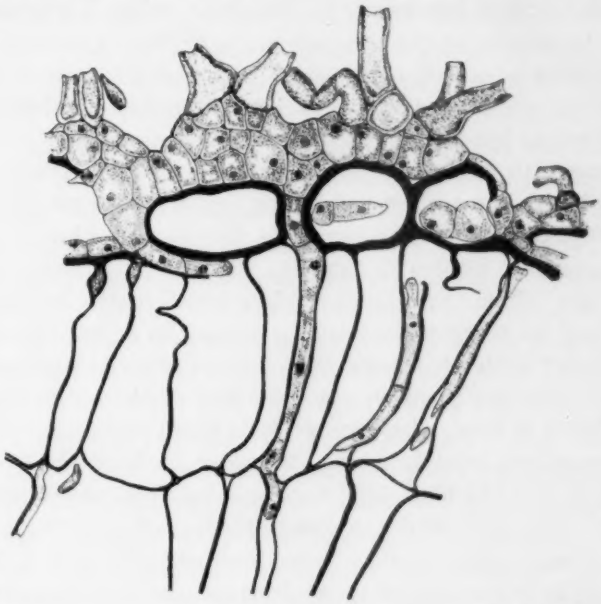

FIG. 2. Hyphae from subcuticular stromatoid layer of *Venturia inaequalis* in the palisade of the leaf. Stromatoid tissues have developed between the epidermal cells, with the results that the walls are ruptured and the fungus enters the cells. Most of the hyphae, however, are intercellular. Camera lucida drawing. (×625.)

contains the ascogonial initial before it has become curved while the one at the left contains an ascogonium which is slightly curved. Indications of a septation may be seen near one end of this ascogonium. Plate V, C represents a perithecium containing an ascogonium with four distinct septations, while D represents one containing an ascogonium with two septations, which are not discernible in the photomicrograph. No indications were found of the presence of trichogynes in any of these cases. It would seem, therefore, that septation of the ascogonium may occur before the trichogyne is formed. Plate V, E represents a perithecium in which one end of the ascogonium is

apparently pushing through the perithecial wall to form the trichogyne. A long trichogyne is shown in Plate V, F.

Frey (4) found that the ascogonium begins to lose its identity soon after an antheridium is applied to the trichogyne. He believed that it segments into many small cells from which the asci are budded. Killian (10), on the other hand, figured the remains of the ascogonium at the base of the perithecium with ascogenous hyphae arising from it. The present writer has noted the remains of this structure in perithecia which clearly showed the presence of ascogenous hyphae. Plate V, H represents a perithecium in which the ascogonium has begun to disappear, while I represents one in which only the remains of the ascogonium are visible. Plate V, J represents a somewhat more advanced perithecium with ascogenous hyphae. Killian (10) figured the ascogenous hyphae as both hooked and clubbed structures. The present writer has seen a few instances of hooked structures; but, as a rule, the ascogenous hyphae are not of any characteristic shape. Some are wedge-shaped with the narrow end in the region of the ascogonium.

At the time the ascogonium begins to disappear the lumen of the perithecium is gradually filled with hyphae. Killian (10) describes the origin of "paraphyses" from the cells of the inner wall of the perithecium. At the stage when the ascogonium takes up a position at the base of the ascocarp, the present writer has noted the presence of short projections apparently coming from any point on the inner wall of the perithecium. Later the entire lumen is filled with hyphae from these processes, which for the most part appear to arise from the inner perithecial wall above the ascogonium (Pl. V, J). If these hyphae originated from the wall of the perithecium they were not true paraphyses. They gradually disappeared from the lumen as the perithecium approached maturity (Pl. V, J, K and Pl. VII, A, B, C). In fully mature perithecia from nature they were rarely visible. If, however, the perithecia were forced to maturity in the laboratory, the hyphae might be found, although they showed signs of disintegration.

In tracing the development of perithecia, stages have been designated as follows: "simple coil" (Pl. V, A, B), extending from the time of the first appearance of perithecial initials until the ascogonium appears as a curved structure; "ascogonial" (Pl. V, B–H), from the time the ascogonium is fully differentiated until it begins to disappear; "filamentous" (Pl. V, I), from the time the ascogonium begins to disappear and the lumen of the perithecium is filled with the hyphae until the ascogenous hyphae appear; "pre-ascus" (Pl. V, J), from the time the ascogenous hyphae appear until the asci begin to appear; "early ascus" (Pl. V, K and Pl. II, A), from the appearance of the asci until the first ascospores are delimited; "delimitation" (Pl. VI, B), from delimitation of the ascospores until they become

olivaceous; "olivaceous" (Pl. VI, C), from the appearance of the first olivaceous ascospores until the first discharge; and "discharge," the first observed ejection of the ascospores. It should be noted that, in some cases, discharge may not take place from perithecia containing olivaceous ascospores even though they are kept moist for a considerable period. This is probably because the ostiole is not open at the time (see Wallace, 15).

Influence of the Time of Leaf-fall in 1924

During the autumn, winter, and spring of 1924–25 a detailed study was made of the development of perithecia in the successive collections of leaves which were taken from the tree and placed on the ground at intervals during the autumn and early winter. A brief preliminary report of this work has been made by Keitt and Wilson (9). A more detailed account follows.

Since the mode of penetration of the fungus from the subcuticular stromatoid layer into the inner tissues of the dead leaf has been described earlier, it will not be taken up here. It is necessary, however, to describe the progress of penetration in relation to the time of leaf-fall. The first fixation from living leaves on the tree, made on October 6, showed that the fungus was present in the leaves as subcuticular stromatoid layers, with no evidence that it had begun to penetrate the lamina. Fixations of apparently living green leaves from a Virginia (crab) tree on October 15 and October 20 showed that in some instances short outgrowths from the stromatoid layer had penetrated between the epidermal cells and a short distance into the palisade layer (Fig. 1). Material fixed prior to November 2, when the leaves were killed by freezing, failed to show any further progress of the fungus into the lamina of leaves which remained on the tree. After this date the fungus penetrated more extensively into the tissues of the dead leaves which remained on the tree, but fixation made as late as December failed to show any evidence of initiation of perithecia in these leaves. Fixations of leaves from the trees of the Wealthy and an unknown variety did not show the fungus in the lamina at any time previous to November 24. After this date it was found commonly in the inner tissues of the dead leaves on the trees.

The results presented in table 1 show that the time of leaf-fall in this year had a marked influence on the time the ascospores were matured the following spring. Unfortunately, owing to the writer's absence from Madison after May 11, 1925, a close study was not made of the maturation and discharge of perithecia in collections made on and after October 7, 1924. It was determined, however, that the initial discharge from these collections took place between May 25 and June 10, 1925. It is noteworthy that the perithecia in leaves of the December 17 collection were mature by June 10.

but that those of the February 9 collection failed to mature even after being placed in favorable moisture and temperature conditions on June 10 and kept thus for three weeks.

Further evidence that the perithecia in leaves which were taken from the tree early in the autumn were more advanced than those in leaves collected later is given in table 2. The time required to bring the ascospores in 100 per cent of the asci to maturity when favorable moisture and temperature were supplied in the laboratory in March varied from 10 days in the August 12 collection to 22 days in the November 10 collection. Under field conditions this difference in time would have been much greater.

TABLE 2.—*Relation of the time of leaf-fall to the maturation of ascospores of Venturia inaequalis, Madison, Wis., 1924–25*

Date of leaf-fall	Percentage of asci[a] which contained olivaceous ascospores on the stated dates of observation:[b]						
	Mar. 21	Mar. 24	Mar. 28	Apr. 2	Apr. 4	Apr. 6	Apr. 9
Aug. 12	30	85	100	100	.....	.....	.....
Aug. 26	1	65	100	100	.....	.....	.....
Sept. 9	0	45	70	90	100	.....	.....
Sept. 23	0	30	50	80	100	.....	.....
Oct. 7	0	0	20	66	80	100	.....
Oct. 31	0	0	12	22	65	80	100
Nov. 10	0	0	2	50	60	90	100

[a] Percentage of the total number of asci observed. The more advanced perithecia were picked from the leaves for these examinations.

[b] Sub-samples of leaves were taken from the stated collections on March 18 and placed in moist chambers at 16° C.

Further analysis of the data collected during the autumn of 1924 indicates that perithecia were initiated at different rates in leaves which fell at intervals during the autumn. When the average daily increase in diameter of perithecia is calculated from (1) the average diameter of the more advanced perithecia at the date of first measurement, and (2) the number of days from leaf-fall to the date of first measurement, the results of column four in table 3 are obtained. It would seem from these data that the most favorable time for initiation and early growth of perithecia during the autumn of 1924 was through the month of September and the early part of October. In 36 days perithecia were produced in the September 23 collection which were of practically the same size and stage of development as the perithecia in the August 12 collection 65 days after leaf-fall. Furthermore, there is a successive increase in the average daily growth of perithecia

in the August 26 and September 9 collections. There was a sudden diminution of average daily growth of perithecia in collections made after September 23. It was found that the perithecia were not initiated in the November 10 collection until three months after leaf-fall. A further discussion of the data in table 3 will be taken up in the section on temperature relations.

TABLE 3.—*Relation of temperature and precipitation to initiation and early development of perithecia of Venturia inaequalis in leaves placed on the ground at intervals during the autumn, Madison, Wis., 1924*

Date of leaf-fall	Days from leaf-fall to first measurement	Diameter of perithecia at first measurement	Average daily increase in diameter of perithecia	Average mean temperature of the 15 days following leaf-fall	Total precipitation of the 15 days following leaf-fall
	No.	Microns	Microns	° C.	Inches
Aug. 12	65	42	*0.6*	*22*	2.58
Aug. 26	64	57	*0.9*	*18*	1.61
Sept. 9	50	49	*1.0*	*15*	1.49
Sept. 23	36	39	*1.1*	*13*	0.40
Oct. 7	56	20	*0.4*	*15*	0.01
Oct. 31	81	30	*0.4*	*7*	2.23
Nov. 10	90	35	*0.4*	*6*	0.50

A very pertinent question arises from the consideration of the data just discussed. Are perithecia initiated and do they develop during mid-winter? From the evidence at hand it would seem that perithecia were initiated during the winter of 1924–25. Fixations of leaves taken from the tree on November 10 and a second fixation from the same collection on December 5 after a period on the ground failed to show any evidence of perithecia. On January 20, however, early stages of perithecia were found to be common in leaves of this collection. The evidence also suggests that growth of perithecia took place, at least in leaves of the later collections (October 31 and November 10). On January 20, 1925, the perithecia in leaves of the October 31, 1924, collection averaged 30 microns in diameter, while on February 12 they averaged 58 microns. On January 20 the perithecia in the leaves of the November 10 collection averaged 38 microns, while on February 12 they averaged 60 microns. The evidence does not suggest such a marked increase in size of the perithecia in the leaves of the August 12 and 26 collections. In these collections it was very infrequent that the average diameter at any one date during mid-winter was found to be greater than the average diameter of the perithecia in the preceding fixation from the same collection. This does not prove, however, that slow changes were not taking place in the perithecia of these collections. It should be borne in mind that the method of obtaining measurements was designed to ex-

clude from the record all but the earlier initiated perithecia of each collection. It is possible, therefore, that perithecia may have been initiated in leaves of these earlier collections during mid-winter.

Column two of table 4 presents data on the number of days from leaf-fall to the first observed ascospore discharge from each collection. It shows that with delayed leaf-fall there was a shortening of the time required to mature ascospores. This phenomenon will be discussed in the section dealing with temperature.

Comparing the dates of initial discharge from leaves of the various collections with the development of the host, we find that the perithecia in the leaves of the August 12 and August 26 collections began to discharge at about the time that such varieties as the Wealthy were in the "pre-pink" or "closed-cluster" stage. The September 9 and September 23 collections began to discharge during the latter part of the blooming period, while the initial discharges did not take place in the later collections until some time after the petals had fallen. It will be noted that olivaceous ascospores were present in the leaves of the August collection on April 13 but the first discharge was not noted until April 22. This was due to a spring drought which retarded the development of perithecia in all of the collections.

Influence of the Time of Leaf-fall in 1925

The results of the examination of perithecia in leaves of the Virginia (crab) variety are presented in columns four and five of table 4. The results from the McIntosh variety agree in the major details with those from the Virginia. There are, however, some significant differences, which will be discussed later.

As was found in 1924–25, the time of leaf-fall was a factor in determining ascospore maturation the following spring. In disagreement with the results of the former year, however, perithecia in leaves of the August collections did not come to maturity in advance of perithecia in leaves placed on the ground in September. The August collections had not matured ascospores by the time the last observation was made on May 25, 1926. Fixations of leaves from these collections showed the presence of perithecia, but some appeared to be abnormal. Others appeared to be normal, yet they did not mature when the leaves were placed in favorable temperature and moisture conditions on May 27 and kept thus for two weeks. This condition was met with in all of the collections made both at Madison and Sturgeon Bay during the month of August. Due to an exhaustion of the leaf material by May 25 these studies could not be pursued farther.

The first collection to mature perithecia was that made on September 16. A dry spring retarded development of the perithecia, and it was not until

TABLE 4.—*Relation of the time of leaf-fall to the time of maturity of perithecia of Venturia inaequalis, Madison, Wis.*

1924–25		1925–26			1926–27		
Date of leaf-fall	Days from leaf-fall to first observed discharge[b]	Date of leaf-fall	Date of first observed discharge[b]	Days from leaf-fall to first observed discharge[b]	Date of leaf-fall	Asci with delimited ascospores on April 7[c]	Asci with mature ascospores on April 7[c]
	No.			No.		Per cent	Per cent
			None by	.....		0	0
Aug. 12	253	Aug. 12	May 25	.....	Aug. 5	0	.....
Aug. 26	243	Aug. 29	Do	.....	.....	.....	0
Sept. 9	244	.....	.....	.....	Sept. 5	10	45
Sept. 23	230	Sept. 16	Apr. 29	225	Sept. 20	71	3
Oct. 7[a]	246	Oct. 19	May 15	208	Oct. 9	18	0
Oct. 31[a]	222	.....	.....	.....	Oct. 25	4	0
Nov. 10[a]	212	Nov. 15	May 15	181	Nov. 8	0	0
.....	.....	Nov. 27	May 25	179	.....	.....	.....
Dec. 17[a]	175	Dec. 13	None by	.....	Dec. 20	0	0
.....	.....	.....	May 25	.....	.....	.....	.....
.....	.....	Jan. 9	Do	.....	Jan. 12	0	0

[a] The initial discharges of ascospores from these collections took place between May 25 and June 10.

[b] Leaves were collected at frequent intervals during the spring and were tested for ascospore discharge by wetting them and placing them in moist chambers over glass slides.

[c] Percentage of the total number of asci observed. The more advanced perithecia were picked from the leaves for these examinations.

April 29 that the first discharge was recorded from the leaves of this collection. The trees of many of the commercial varieties of apples were in the late "closed-cluster" stage at this time. The perithecia in the October 19 and November 5 collections came to maturity at the time the apple trees were in full bloom, while the perithecia in the leaves of the November 27 collection did not begin to discharge until the petals were off of most varieties.

In conformity with the results of the previous year, the data presented in column five of table 4 show that, with delayed leaf-fall, there was a shortening of the time required to mature ascospores. The November 27 collection was placed on the ground 72 days later than the September 16 collection, yet it matured ascospores only 26 days later than that collection. This further emphasizes the ability of the fungus to produce mature perithecia in a shorter time under certain conditions than under others.

It was stated earlier that the results obtained concerning the development of the perithecia in leaves of the McIntosh variety were in general agreement with those from similar studies of the Virginia (crab). This is true in the sense that a delay in leaf-fall was correlated with a delay in the time of ascospore maturity. On the other hand, the perithecia in the McIntosh leaves were constantly less advanced than those in the parallel Virginia collections. For example, when the perithecia in the September 16 Virginia collection contained asci of approximately mature size, the corresponding McIntosh collection contained perithecia with asci just beginning to form. Further studies concerning the development of perithecia in leaves of different varieties will be discussed later.

Influence of the Time of Leaf-fall in 1926

Columns seven and eight of table 4 contain results from examinations of the Virginia leaves. The results from the leaves of the unknown variety were comparable in all major respects with those from the leaves of the Virginia. Due to the opening of the field laboratory early in the spring the writer found it necessary to leave Madison before the perithecia in all of the collections had matured. The results are sufficient, however, to show that the time of leaf-fall in the autumn of 1926 influenced the time ascospores were matured the following spring. In conformity with the results of the preceding year the collection made in mid-September was the first to mature ascospores. This suggests, as did the results of both 1924–25 and 1925–26, that September was much more favorable than August for initiation and early development of perithecia.

Ascospores were mature in the orchard at Madison in 1927 by March 14, the earliest date recorded during the three seasons that the present work

has been under way. While the September 20, 1926, collection had mature ascospores by this date, the initial discharge was not recorded until April 5. At this time the buds of all commercial varieties had begun to swell but no susceptible tissue was exposed. The perithecia in the October 9 collection began to eject ascospores at the time most of the varieties of apples were in the "green-tip" stage. The September 5 and October 25 collections contained perithecia with mature ascospores at the time the trees were in the green-tip stage, but no discharge was recorded up to the time the trees were in the closed-cluster stage. No perithecia had become erumpent on the leaves of the August 5, December 20, or January 12 collections by April 22, at the time the host was in the closed-cluster stage.

It is readily seen that comparisons between the stage of advancement of the host and the time of maturation of the perithecia do not reveal such marked differences due to delay in leaf-fall as in 1924–25 or 1925–26. This was because the host developed very slowly while perithecia developed more rapidly. If, however, the number of days between the maturation of the perithecia in the various leaf-fall collections is considered, the differences are more striking. For example, the perithecia in the leaves placed on the ground on October 25, 1926, did not mature perithecia until 17 days after the first observed discharge of ascospores from the collection made September 20, 1926. It is in years such as 1927, when the host passes slowly through the period of bud unfolding, that the effects of delayed leaf-fall are minimized.

Factors which Influence the Time of Leaf-fall

Since it has been shown that early leaf-fall contributes to early maturation of perithecia, a knowledge of the factors which influence the time of leaf-fall should be of value. While a comprehensive study of this subject is beyond the scope of the present paper, the following observations have been made.

Meteorological conditions. The time of leaf-fall may be influenced by temperature and moisture in the autumn. In some instances the first freeze that is sufficient to kill the leaves may lead to retention of the foliage, while in others it may hasten defoliation. In the autumn of 1926, for example, the writer noted that trees of the Plum Cider variety lost all of their foliage within three days following the first killing freeze, while defoliation of the Northwestern Greening appeared to be checked.

Cultural practices and soil. Trees which are in good condition appear to retain their foliage longer than those in poor condition. In an effort to determine whether the application of commercial fertilizer would be practicable in delaying leaf-fall, sodium nitrate was applied to trees of the

Dudley, Wealthy, and Fameuse varieties during the growing seasons of 1925 and 1926. The results of these tests are as yet inconclusive.

Scab disease. That apple trees which are heavily infected by the scab become defoliated earlier than trees of the same variety with little leaf infection was noted by the writer in the autumn of 1926. The unsprayed Dudley trees in the experimental plots at Sturgeon Bay had lost 100 per cent of their foliage by October 27, while the sprayed trees had lost only 55 per cent.

Variety of apple. Ewert (3) reports observations on the time of leaf-fall among different varieties of apples. He states that the varieties which are most severely attacked by the scab fungus lose their leaves much earlier than those which are more resistant to the disease. He also states that the earliness or lateness of leaf-fall is a varietal characteristic. In a year when scab infection was very rare he found that the "Virginischer Rosenapfel," which is said to be very susceptible, lost its leaves much earlier than the "Antonowka," which is said to be resistant.

The present writer has made observations in the commercial apple orchards of Sturgeon Bay during two seasons when, owing to efficient spraying methods and unfavorable conditions for infection, severe leaf infection was very rare. The results, which appear in table 5, show that certain

TABLE 5.—*Records of the relation of apple varieties*[a] *to the time of defoliation, Sturgeon Bay, Wis.*

Varieties	1925	1926
	Defoliation on Nov. 21	Defoliation on Oct. 27
	Per cent	Per cent
Grimes Golden	5	—
Stayman Winesap	10	—
McIntosh	15	5
Fameuse	30	10
McMahon	80	15
Northwestern Greening	90	20
Delicious	95	5
Wealthy	95	20
Virginia (crab)	98	—
Duchess	100	40
Dudley	100	55
Lubsk's Queen	100	80

[a] It was not possible to make all these observations in one orchard. Consequently, other factors than varieties may have played a part in occasioning the differences noted. The same trees were observed in 1925 and 1926.

varieties consistently became defoliated before others. In a small orchard at Madison, planted to nine varieties of the same age, the McIntosh, Fameuse, and the unknown variety mentioned earlier, have consistently held their leaves later than such varieties as the Wealthy and Plum Cider, regardless of the severity of leaf infection. The unknown variety has retained many of its leaves until January. The crabs appear to have a tendency to become defoliated earlier than many other varieties, although this may be due in part to severe leaf infection.

RELATION OF VARIETY OF APPLE TO THE PRODUCTION OF THE ASCIGEROUS STAGE

A consideration of the development of the ascigerous stage in leaves of different varieties of apples is important from the standpoint of possible control methods dealing with the limitation of the primary inoculum. If it should be found that certain varieties are constant sources of inoculum while others produce little or no perfect stage, special attention could be given to the offending varieties.

It is readily apparent that there is a possibility that several factors may contribute towards making certain varieties important sources of ascosporic inoculum. Of these, susceptibility to leaf infection, the disposition to early leaf-fall, and the character of the leaves themselves, as they may favor or inhibit the development of the perithecia, seem to be among the more important. It has been shown earlier that the amount of leaf infection bears an important relation to the quantitative aspects of perithecial production. It has also been shown that the time of leaf-fall has an important bearing on the time the ascospores mature in the spring. As far as the writer is aware, there has been no comparative study, hitherto, of production of perithecia in leaves of different varieties of apples. Schneiderhan and Fromme (13) report studies in which they compared the discharges of ascospores from leaves of the Rome, Stayman, and Winesap varieties throughout one season. They found no significant differences in the amount of ascospores discharged from the leaves of the three varieties. The present work on this subject has been confined to testing varieties in relation to the early and abundant development of ascospores.

Methods

Leaves were gathered from a number of varieties of apple with as comparable amounts of leaf infection as it was feasible to obtain. While the collections of each year were not all made on the same date, they were made within a few days and are closely comparable. The leaves were put in cloth bags of the type mentioned earlier and placed in the orchard for overwintering. In the spring, sub-samples were taken from each variety and placed

in moist chambers at 16° C. At intervals, 15 to 20 of the more advanced perithecia were picked from the leaves of each variety and examined. Data were gathered on the production of mature perithecia in leaves of the different varieties and on the time required for maturation of perithecia in leaves of each variety. Data were also taken on the relative abundance of the perithecia in leaves of the different varieties, but these data are not considered entirely reliable since the difference in the amount of leaf infection might have influenced the results. Table 6 contains a summary of the results obtained from these experiments.

Results

An experiment in 1924 indicated that the Charlemoff and a seedling ("Talent") were much later in maturing perithecia than the Virginia (crab), the Wealthy, or the unknown variety (mentioned earlier). Unfortunately, the available trees of the two former varieties had no leaf infection the following two seasons; and, consequently, they could not be included in the experiments of those years.

The experiments of 1925–26 and 1926–27 emphasize what seems to be a consistency of the Virginia (crab) in maturing perithecia before many of the other varieties. This was also true of the Shields (crab) in 1925–26. The Martha (crab) was somewhat later than the Virginia in both years. The results are not consistent for the Wealthy and Fameuse. In 1925–26 they both matured perithecia at the same time as the Virginia, but in 1926–27 they were considerably behind that variety. The Northwestern Greening, McMahon, and the Plum Cider were somewhat later than the Virginia in both years. It was stated earlier that the McIntosh leaves used in the leaf-fall experiments of 1925–26 were later than the Virginia (crab) in maturing the perithecia. An examination of table 6 will show that this also seemed to be true in 1926–27. All varieties under observation in the two latter years of this work produced mature perithecia in nature.

While the two years' data presented here are not sufficient to warrant any definite conclusions, they strongly suggest that perithecia are developed more rapidly in leaves of certain varieties than in others. Through experience the writer has learned to look in the leaves of the Virginia (crab), Shields (crab), and Dudley for the first mature perithecia in the spring. Besides being easier to locate on the glaucous leaves of the crabs, the perithecia are much more abundant there than on leaves of most other varieties. Although the dorsal surfaces of the young Dudley leaves are pubescent, yet it is not difficult to find the perithecia when they become erumpent. Likewise there is no great difficulty in finding the perithecia on the pubescent dorsal surfaces of the McIntosh leaves after they break through the epidermis.

TABLE 6.—*Relation of variety to the production of the perfect stage of Venturia inaequalis in apple leaves, Madison, Wis.*

Variety	Experiment of 1925–26		Experiment of 1926–27		
	Asci with mature ascospores after:[a]		Asci with mature ascospores after:[b]		
	Three days' forcing	Eleven days' forcing	Seven days' forcing	Eleven days' forcing	Fifteen days' forcing
	Per cent[c]	Per cent[c]	Per cent[c]	Per cent[c]	Per cent[c]
Virginia (crab)	40	100	32	60	90
Powers (crab)	0	65	50	50	80
Martha (crab)	0	20	0	29	50
Shields (crab)	28	50	—	—	—
Whitney (crab)	0	0	—	—	—
Wealthy	43	94	0	0	0
N. W. Greening	0	100	0	0	18
Patten Greening	—	—	0	0	0
Fameuse	49	75	0	0	0
McIntosh	—	—	0	0	7
McMahon	0	50	0	0	0
Dartt's Hybrid	—	—	30	67	80
Winter Banana	—	—	0	26	59
Plum Cider	0	0	0	0	0
Arkansas Black	—	—	0	0	15
Babbitt	—	—	0	12	40
Windsor	—	—	0	0	0

[a] Sub-samples of collections taken from the tree on October 10, 1925, and overwintered on the ground, were brought into the laboratory on April 11, 1926, and subjected to favorable temperature and moisture conditions for maturation of perithecia.

[b] Sub-samples of collections taken from the tree on October 5–11, 1926, and overwintered on the ground, were brought into laboratory on February 23, 1927, and subjected to favorable temperature and moisture conditions for maturation of perithecia.

[c] Percentage of the total number of asci observed which contained olivaceous ascospores. The more advanced perithecia were picked from the leaves for these examinations.

RELATION OF TEMPERATURE TO THE DEVELOPMENT OF THE ASCIGEROUS STAGE

It was evident from a consideration of the influence of the time of leaf-fall on the development of the perfect stage that this was not the only factor which was concerned in inducing variability in the time of maturity of ascocarps. If the time element alone were concerned, one would expect that leaves placed on the ground two months apart would mature ascospores two months apart. That this was not the case is clearly shown in table 1. Furthermore, leaves placed on the ground at different times during the autumn of 1924 did not initiate perithecia at the same rate, as is shown in table 3. It was with these facts in mind that the influence of temperature on the development of perithecia was studied.

The literature on temperature in relation to the development of the ascigerous stage of *V. inaequalis* is very fragmentary and inconclusive. In his work on the *Venturia* on *Pyrus coronaria*, Jones (7) made some observations on the production of perithecia in artificial media. He found that they were produced at 10°, 16° and 26° C. Howitt and Evans (6), through observational data, sought to correlate the development and the time of initial discharge of ascospores in the spring with temperature and precipitation. They conclude that, during the six seasons they were concerned with, the average mean temperature of January, February, and March seemed to bear a more direct relationship to the development and time of discharge of the ascospores than did the amount of precipitation from April 1 to the date of first discharge. They further report that the average mean temperature of January, February, and March was more closely related to the development of perithecia and the time of discharge than the mean temperature of the days of April and May preceding primary discharge. Under controlled temperature conditions, Keitt and Jones (8) found that ascospores matured slowly in leaves kept at 4° C. and at 7° C., but there was a successive increase in the rate of maturation at 12° and 16° C., with an optimum near 20° C. At 24° C. they found that maturation was definitely checked. They also found that discharge of ascospores occurred at temperatures ranging from 0.5 to 32° C.

Methods

The present work has been carried out with the aim of subordinating all factors other than temperature. The results have been obtained by subjecting the fungus to controlled temperatures while growing in pure culture and in naturally infected dead leaves.

The fungus has been grown on oatmeal, apple-leaf, malt, apple-peel, synthetic, potato dextrose, and cornmeal agars. Preference has been given to the oatmeal and apple-leaf agars, for reasons which will be discussed

later. The oatmeal agar was made from 50 gms. of rolled oats and 15 gms. of agar a liter. The apple-leaf agar was made by steaming 100 gms. of dead apple leaves in 1,000 cc. of water for 30 minutes, passing the decoction through filter paper and adding 15 gms. of agar. The methods have been standardized as far as possible. Petri dishes of uniform size were poured with 15 cc. each of the desired medium. After the agar had solidified the plates were treated with 0.5 cc. each of a conidial suspension from a 10- to 12-day-old culture of the organism. The suspension was allowed to spread over the agar as evenly as possible and the cultures were incubated at room temperature. When the fungus had become macroscopically visible on the agar, and before perithecia had begun to form, the petri dishes were placed in triplicate at the desired temperatures. Control was effected within 1° C. To avoid excessive desiccation at the higher temperatures, the petri dishes were placed in large glass chambers with moist absorbent cotton. After the cultures had been kept at the controlled temperatures for from 5 to 10 days, depending on the medium used, data were taken on the abundance and the diameters of the perithecia in each plate. The number of perithecia was counted in 100 fields of the microscope (a microscopic field was 2.4 square millimeters in area). The observations were made in areas in which the fungus occurred in abundance. Measurements were made of 50 perithecia in each plate when that number could be found.

In the studies of the effect of temperature on initiation and early development of perithecia in leaves, the leaves were taken from the tree before the fungus had grown below the stromatoid layer. Several of these leaves were cut into pieces and one piece from each was placed in a moist condition at each of the desired temperatures. After 10 to 16 days the leaves were removed and cleared by the chloral hydrate-potassium hydroxide method mentioned earlier (Peace, 12). Measurements were then made of 100 perithecia in the leaves kept at each temperature.

The leaves which were used for the effect of temperature on the growth of asci and delimitation and maturation of ascospores were from a supply which had been taken from the tree on a certain day in the autumn and placed in the orchard until needed. When the asci began to appear, a number of leaves were selected which bore the diffuse type of lesions scattered uniformly over the surfaces. These leaves were cut into pieces and one piece from each placed in a petri dish and kept at the desired temperature. The moisture was controlled, as nearly as possible, by a standard method of wetting. Examinations of the perithecia were made at intervals by picking a number of the more advanced ones from each piece of leaf and crushing them in water on a glass slide. Records were made of the percentage of asci which were three-fourths mature size or larger (including those with delimited and mature ascospores), the percentage with delimited ascospores

(including those with mature ascospores), and the percentage with mature ascospores. In these results and those shown in the other tables and graphs, the writer wishes to interpret the quantitative aspect very conservatively. While an earnest attempt has been made to apply quantitative methods as fully as feasible in the collection and presentation of data, the nature of the problems is such that the results are best considered as semi-quantitative. The purpose, in the main, has been to show trends and relationships with accuracy adequate for the purposes served, rather than to seek strictly quantitative data on matters too complex to be treated in a rigidly quantitative way in the present state of our technique and resources.

Growth of the Fungus into the Interior of the Dead Leaf

Experiments have shown that the hyphae of *V. inaequalis* will grow from the subcuticular stromatoid layer into the lamina of the leaf within three days at temperatures ranging from 4° to 28° C., but no growth was found in the leaves which had been kept in a moist condition for one week at 31° C. Fixations of leaves which had been kept at −4° C. for 20 days showed an occasional hypha in the interior. An examination just before this experiment was begun showed no hyphae in the interior of the leaves. To avoid mistaking the hyphae of other fungi for those of *Venturia*, no hypha was considered to be that of *Venturia* unless it was seen to come directly from the stromatoid layer. The characteristic conidiophores identified the stromatoid layer of *Venturia*.

There was a distinct difference in the character of the hyphae in the leaves kept at the different temperatures. Whereas, the hyphae in the leaves which were kept at 4°, 10°, and 16° C. were comparatively thin-walled and of light color, those in the leaves which were kept at the higher temperatures (21°, 24°, and 28° C.) were dark olivaceous to brown in color and moniliform in shape. On certain solid media similar effects of temperature were noted. At 28° C. the hyphae were very slender and often grew in a sinuous fashion through the substratum.

Initiation and Early Growth of Perithecia

The growth of the fungus in culture is only approximately similar to its growth in the leaf after leaf-fall. Most media which are favorable for vegetative growth of the fungus are also favorable for production of conidia. The abundant production of conidia undoubtedly has a tendency to delay initiation of perithecia. Furthermore, the fungus responds differently to temperature when growing in different media. On malt agar, for example, an increase of temperature to 24° C. was found to be accompanied by an increase in the production of mycelium and of sterile aerial hyphae. When

the fungus was exposed to temperatures ranging from 4° to 28° C. it was found that the greater number of conidia was produced at 10°, 13°, and 16° C., with an apparent sharp diminution in production at 19° C. This example is presented for the reason that in two experiments on this agar the perithecia were produced in greater numbers at 19° C. Had malt agar alone been used, the optimal temperature for initiation of perithecia would have appeared to be near 19° C. When the fungus was grown on agar made from dead apple leaves, on the other hand, temperature did not appear to have so marked an effect on vegetative development as was the case when it was grown on malt agar. It is noteworthy that comparatively few conidia were produced on this agar and their abundance was not affected to a noticeable extent by the range of temperature used. Most of the experiments on the relation of temperature to initiation and growth of perithecia were carried out on this medium.

The growth of the fungus on oatmeal agar more closely paralleled that on agar made from dead apple leaves than did the growth on malt agar. While conidia were produced on oatmeal agar, their abundance did not vary markedly at temperatures between 7° and 21° C. Some of the experiments on the effect of temperature on the initiation and early growth of perithecia were carried out on this agar for comparison with the results from apple-leaf agar.

Figure 3 is a graphic representation of results obtained when the fungus was grown in apple-leaf agar, oatmeal agar, and in naturally infected dead leaves. With the exception of the results from malt agar, all experiments indicate that the optimal temperature for initiation and early growth of perithecia lies between 13° and 16° C. In both leaves and apple-leaf agar the optimum appears to be nearer 13° C. In oatmeal agar, on the other hand, some of the experiments indicated that the perithecia were produced in greater abundance and were of larger size at 16° C., while in others they were more numerous at 13° C. All the data presented in figure 3 agree in one respect; *viz.*, that there was a sharp diminution of initiation and early growth of perithecia at 19° C.

The data in table 3 indicate that perithecial initiation and early growth proceeded more rapidly in leaves which were placed on the ground in September than in those which were similarly placed either before or after September. The meteorological data presented in figure 4 shows that there was a gradual decrease of the mean temperature as autumn progressed. When the mean temperature of the 15 days following each of the leaf-fall collections is calculated and the average daily increase in diameter of perithecia is compared, as in columns four and five of table 3, it is seen that the mean temperatures following the September 9 and September 23 collections are distinctly within the optimal range found for initiation and

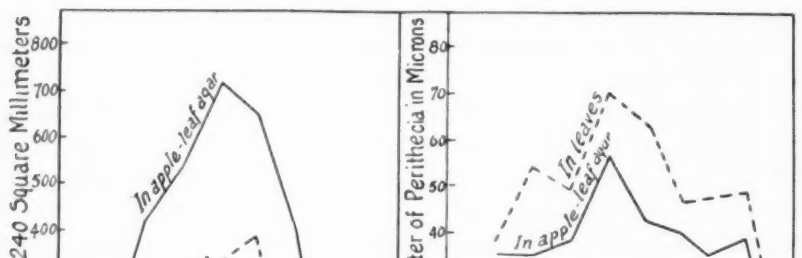

FIG. 3. Relation of temperature to the initiation and early development of perithecia of *Venturia inaequalis* in pure culture and in naturally infected apple leaves.

early growth of perithecia in culture and in leaves. The low daily increase in diameter of perithecia recorded for the October 7 collection cannot be attributed to the temperature immediately following the collection of these leaves, since it is within the optimal range. The rainfall, on the other hand, probably accounts for the difference. Whereas the September 23 collection was followed within 15 days by 0.40 inch of precipitation in four rains, there was only 0.01 inch of precipitation within 24 days following the October 7 collection. The low temperatures prevailing after the October 31 and November 10 collections were placed on the ground probably account for the low daily increase recorded for these collections.

Additional data presented in table 7 show what seems to be a correlation between the temperature following leaf-fall and the initiation and early development of perithecia. It will be seen that in both 1925–26 and 1926–27 the most advanced perithecia were found, when examinations were made in the spring, in leaves which were placed on the ground in September. In both of these years the mean temperatures for the 15 days following these collections were nearer the optimal temperature found for the initiation of perithecia under controlled conditions than were the mean temperatures for similar periods following the August collections. Both high temperatures and lack of moisture (Fig. 4) may have retarded the perithecia in the August collections of 1925 and 1926. On the other hand, the rainfall for the 15 days following the September 5, 1926, collection, although not quite as abundant, was more evenly distributed than the rainfall for a similar period following the September 20, 1926, collection, yet the peri-

thecia in the September 20 collection were distinctly more advanced than those in the September 5 collection. It appears, therefore, that in this case temperature was the determining factor.

Two experiments were conducted during the autumn of 1926 in which leaves were taken from the tree and samples were placed in a moist condition at 4°, 10°, 16°, and 24° C., respectively. After 15 days in one experiment and 20 days in the other the leaves were removed from the respective temperatures and placed in the orchard. In the spring the samples were taken from the orchard and placed in moist chambers at 16° C. In the first experiment, perithecia in the leaves which had been kept for 15 days at 10° and 16° C. came to maturity only slightly ahead of the perithecia in those which had been kept at 4° and 24° C. In the second experiment, however, in which the leaves had been kept at the several temperatures for 20 days, the perithecia in those kept at 10° and 16° C. were distinctly more advanced than those in leaves kept at either 4° or 24° C.

In order not to overestimate the direct effects of temperature on perithecial development in naturally infected dead leaves, it should be borne in mind that high temperatures are favorable to development of various saprophytic organisms in the leaves, provided the moisture conditions are favorable. The leaves which were placed on the ground in August were almost always in more advanced stages of disintegration in spring than those which were placed on the ground later in the season. The rapid disintegration of the leaves might possibly have influenced the development of *Venturia* in those leaves. The direct effect of temperature is, however, of importance. High temperatures seemed to have a tendency to induce aberrant development of the perithecia, especially if abundant moisture was present. In pure culture, the perithecia produced at temperatures of 21° and 24° C. were more irregular in shape than those produced at 13° or 16° C.

The evidence given earlier suggests that initiation and early growth of perithecia occurred between December 5 and January 20, 1924–25. A study of the thermograph records which were taken between these two dates at the place where the leaves were overwintered showed that the temperatures at the surface of the ground beneath the snow remained constantly below 0° C. Similar records made during the winter of 1926–27 show that during December, January, and February the temperature at the surface of the ground beneath the snow seldom fell below −5° C. During the middle of the day the temperature often rose to −1° or −2° C. When the ground was bare, however, considerably lower temperatures were recorded. Under these conditions, of course, the temperature fluctuated much more than when the ground was covered by snow. During the middle of some of the warmer days, temperatures above 0° C. occurred. To determine

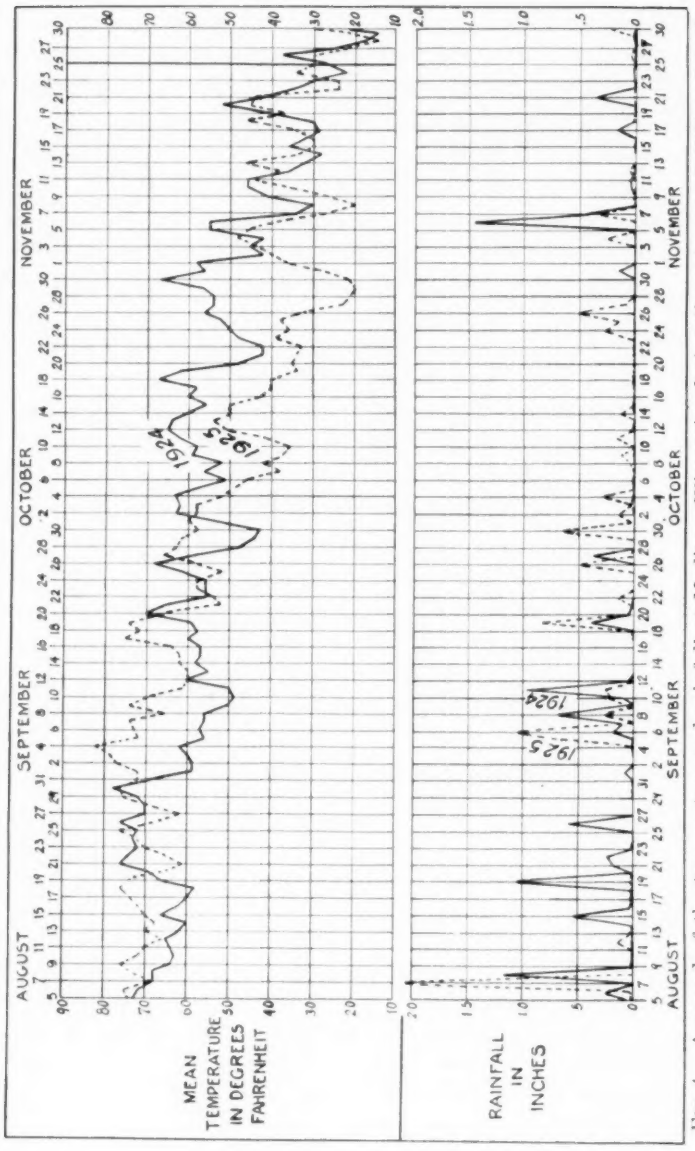

FIG. 4. A record of the temperature and rainfall at Madison, Wisconsin, during the autumns of 1924 and 1925.

TABLE 7.—*Relation of the temperature following leaf-fall to the development of perithecia of Venturia inaequalis, Madison, Wis.*

1925–26			1926–27		
Date of leaf-fall	Mean temp. of the 15 days following leaf-fall	Stages[a] of development of perithecia on April 23, 1926	Date of leaf-fall	Mean temp. of the 15 days following leaf-fall	Stages[a] of development of perithecia on March 7, 1927
	°C.			°C.	
Aug. 12	22	None erumpent	Aug. 5	21	None erumpent
Aug. 29	23	do	Sept. 5	17	Early ascus
Sept. 16	17	Delimitation	Sept. 20	15	Delimitation
Oct. 19	0	Early ascus	Oct. 9	9	Early ascus
Nov. 5	2	None erumpent	Oct. 25	6	do

[a] Stages of development: None erumpent = no perithecia erumpent on the surface of the leaves; Early ascus = asci just beginning to appear; Delimitation = ascospores visible but not yet olivaceous.

whether perithecia would be initiated and develop at temperatures which were kept constantly below 0° C., leaves were collected from the tree in the autumn of 1926 before they had been killed by frosts. Free-hand sections of these leaves showed that the fungus had not penetrated beyond the subcuticular position. The leaves were moistened and placed in an ice-mold of a refrigerating machine. The temperatures in this mold averaged −4° C. and never went above 0° C. In January, during repair of this machine, workmen removed the leaves from the ice-mold. It was not until about eight days later that the writer discovered that they had been removed. They were by this time air-dry. Some tests showed that leaves left under the conditions that these leaves were subjected to during the eight days outside the ice-mold would become air-dry within three or four days. Upon making free-hand sections of the leaves which had been in the ice-mold, it was found that the fungus had penetrated to the interior abundantly and that perithecia at the early stages of fertilization were not uncommon. Unless it can be assumed that the fungus is able to grow into the interior of the leaf and initiate perithecia within five or six days, it must be concluded that at least some part of this development occurred at temperatures below 0° C. The experience of the writer has been that, under the most favorable temperature and moisture conditions, comparatively abundant ramification of the hyphae in the lamina of the leaf takes place only after three or four days. The evidence, reported under the preceding heading, that a few hyphae were found in leaves which had been kept for 20 days at −4° C., lends weight to the probability that growth of the fungus occurred in the ice-mold. Further experiments, however, are necessary to establish this point definitely.

Some experiments indicated that, on certain media, initiation of perithecia was stimulated when the cultures were exposed for a short period to temperatures near 0° C. Petri-dish cultures of *V. inaequalis* on oatmeal and apple-peel agars were prepared as described earlier. When the fungus became macroscopically visible, two sets of three plates each were placed at −4°, 4°, and 21° C., respectively, and one set was placed at 12° C. After three days one set from each of the −4°, 4°, and 21° C. temperatures was transferred to a temperature of 12° C. Examinations of the cultures were made at intervals and the time of appearance and abundance of perithecia were noted. It was found that perithecia appeared in a shorter time and in greater abundance in the plates which had been kept for three days at −4° and 4° C. than in similar plates which had been kept constantly at −4°, 4°, 12°, or 21° C., or in the plates which had been kept for three days at 21° C. In one experiment two plates were placed out-of-doors for 15 hours during a night when the temperature reached a minimum of −18° C.

Upon subsequent removal to a temperature of 15° C., perithecia appeared in greater abundance than in similar plates which had been kept constantly at 15° C. When these experiments were repeated with the fungus growing on agar made from dead apple leaves, no such indications were found of a stimulatory effect of short exposures to low temperatures. On the contrary, the short exposures retarded initiation of perithecia.

Growth of Asci and Delimitation and Maturation of Ascospores

Figure 5 is a graphic representation of the results obtained from one of the experiments on the influence of temperature on the maturation of ascospores in apple leaves. It shows that there were successive increases in the rate of maturation at 4°, 7°, 10°, 13°, 16° and 19° C. The rate of maturation was almost maintained at 21° C., but at 24° C. there was a distinct decrease. At the latter temperature the contents of the asci had undergone disintegration. In some instances the ascospores had been delimited but apparently no wall had been formed around them, with the result that the apical spore often effectively plugged the ascus. Attempts at forcing discharge from such perithecia met with but little success. Additional experiments in which leaves were kept at the higher temperatures for eight days showed that temperatures near 24° C. and above were deleterious to the normal maturation of ascospores if the leaves were in a moist condition, but no marked effect was noted within a similar period when the leaves were kept air-dry while exposed to the high temperatures.

It is noteworthy that the curve represented in figure 5 corresponds very closely to that obtained by Keitt and Jones (8) for germination of ascospores in water. It also conforms, to a marked degree, with their results on the temperature relations of the fungus during infection and incubation in that the optimum in all of these cases appears to be near 20° C.

It was noted earlier that in 1924–25 and 1925–26 with a delay in leaf-fall there was a shortening in the time between leaf-fall and the first discharge of ascospores (Table 4). There were 253 days between the time the collection was placed on the ground on August 12, 1924, and the time the first discharge was observed from this collection; whereas only 175 days elapsed between the time the December 17, 1924, collection was placed on the ground and the time the first discharge was observed. The magnitude of the difference and the consistency of the trend of the data warrant the conclusion that some factor other than time of leaf-fall played a rôle in determining the time necessary for the maturation of ascocarps. Since the data in table 4 show a consistent trend in one direction, it would be expected that the factor involved would likewise show a consistent trend. Since the amount and distribution of rainfall fluctuated greatly, it could

hardly be expected that it might be concerned in inducing the variability mentioned above. Temperature, on the other hand, had a constant trend downward during the autumn months and a constant trend upward during the spring. The explanation of this phenomenon was sought, therefore, through a study of the influence of temperature on development of perithecia at stages preceding maturation. It will be recalled that the optimal temperature for initiation and early growth of perithecia appeared to be near 13° C., while the optimum for maturation of ascospores was near 20° C. This means that there was a shift from a lower to a higher range of thermal requirements. This shift could take place in either of two possible ways: (*1*) a sudden change from the lower to the higher requirement, or (*2*) a gradual shift. What are the effects of the transition from a lower to a higher optimal thermal requirement during a time when the temperatures are gradually declining, and what is the result when the temperatures are gradually rising during the spring? An attempt was made to answer these questions through a study of the effect of temperatures between 4° and 16° C. on the growth of asci and delimitation and maturation of ascospores. Leaves bearing perithecia in the early stages of ascus formation were cut into pieces and one piece from each leaf was kept in a moist condition at 4°, 7°, 10°, 13°, and 16° C., respectively. At frequent intervals several of the more advanced perithecia were picked from the leaf fragments at each temperature. The perithecia were crushed in water on

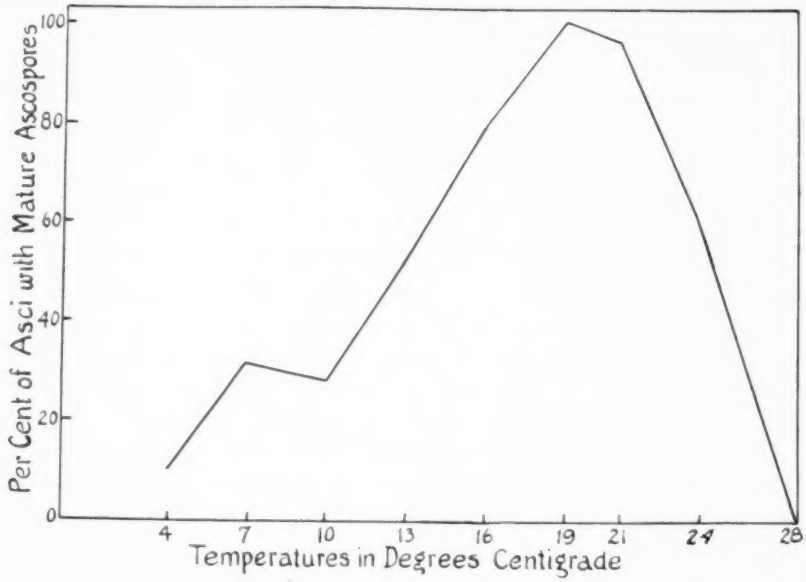

FIG. 5. Relation of temperature to the maturation of ascospores of *Venturia inaequalis* in naturally infected apple leaves.

a glass slide and counts were made of the total number of asci in each perithecium. Records were then made of: (*1*) the number of visible asci which were three-fourths mature size or larger, including those which contained delimited ascospores and those which contained mature ascospores; (*2*) the number of asci which contained delimited ascospores, including those which contained mature ascospores; and (*3*) the number of asci which contained mature ascospores. On the basis of the total number of asci observed, the percentage of asci which were three-fourths mature size or larger, the percentage of asci with delimited ascospores, and the percentage of asci with mature ascospores were calculated. Figure 6 is a graphic representation of the averages of the results of three observations from the same experiment.

The results of three experiments agree in all major aspects. Whereas the curve representing ascus growth rises rapidly with additional increments of temperature above 4° C., the curve representing maturation of asci remains low at 4°, 7°, and 10° C., but rises rapidly at 13° and 16° C. In addition to this, it was found that at 4° C. 85 per cent of the asci became three-fourths mature size or larger in eight days, while no mature ascospores were produced in that time. At 7° C. 98 per cent of the asci became three-fourths mature size or larger in eight days, while only 2 per cent of the asci matured ascospores. In the three experiments the curve representing delimitation of ascospores also showed a lag at the two lowest temperatures at the time the first observations were made. The average of three observations from one experiment, however, gave a curve which was intermediate between the curve representing maturation and that representing ascus growth (Fig. 6). This suggests that the temperature requirements for delimitation were intermediate between those for ascus growth and maturation of ascospores; but, since the other two experiments were not carried farther than the first observation, conclusive evidence on this point is lacking. In any case, the results of the three experiments are in agreement in that growth of asci takes place more readily at low temperatures than does maturation of ascospores. The writer considers this to be evidence of a gradual change in thermal requirements as the asci approach maturity.

If, as indicated by the results presented in figures 3, 5, and 6, the optimal range of temperature for initiation and early growth of perithecia is distinctly lower than that for maturation of ascospores, and the transition from the lower to the higher range takes place gradually as the perithecia develop, a possible explanation is afforded of how temperature may have been concerned in inducing the variability which was found in the time necessary for development of the perithecia in the leaf-fall collections of both 1924–25 and 1925–26 (Table 4). The perithecia in the leaves which

had been placed on the ground in early autumn (September), having reached an advanced stage, were checked in their development by the gradually declining temperatures of late autumn. The perithecia in the leaves which had been placed on the ground later in the autumn, on the other hand, being less advanced, and having a lower thermal requirement, were able to develop at a greater rate for a longer time in the late autumn than the more advanced perithecia. Not only this, but the less advanced perithecia would respond more quickly to the gradually rising temperature of spring. The evidence which was reported earlier suggests a further possible factor which may have contributed to this variability; *viz.*, growth of the less advanced perithecia during midwinter. This possibility, however, lacks conclusive proof.

Under Wisconsin conditions, the fact that the higher thermal requirement has not been entirely reached by the time the asci appear is a distinct advantage to the fungus. The occurrence of temperatures a few degrees above 0° C. during late winter or early spring is accompanied by development of the ascogenous elements. This places the asci in such condition that only a few days of temperature above 10° C., with sufficient moisture, are necessary to permit maturation of ascospores. It can be seen how a

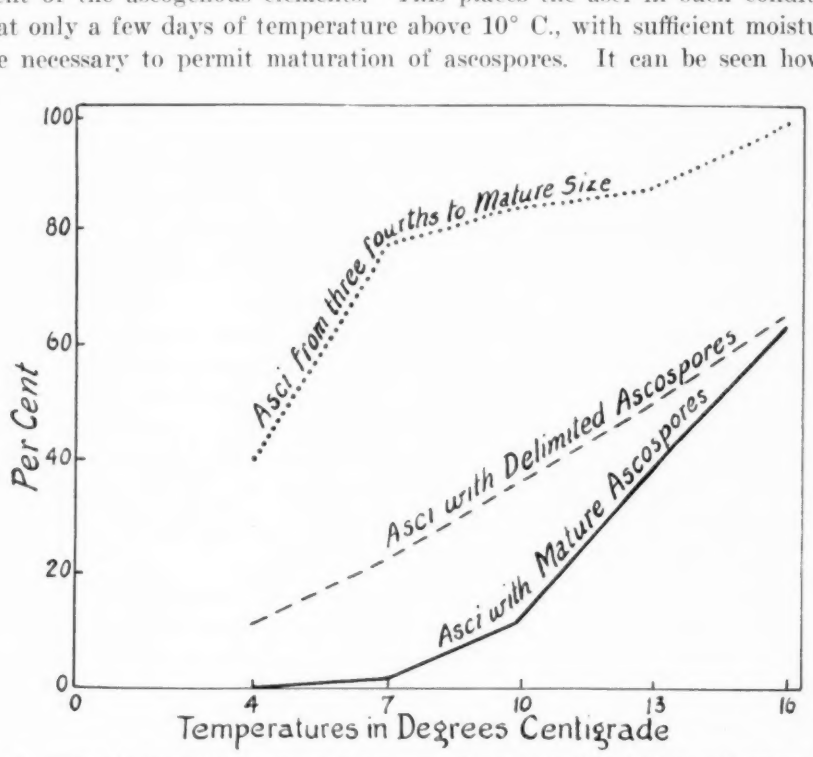

FIG. 6. The relation of temperature to ascus growth and the delimitation and maturation of ascospores of *Venturia inaequalis* in naturally infected apple leaves.

relatively short period of favorable temperature during the spring might lead to more development than would occur over a longer period during which the temperature was slightly below the critical range. An example of the rapid development of perithecia during a short period of favorable temperatures was witnessed by the writer at Madison in the spring of 1927. By the end of February the most advanced perithecia in nature were at about the "pre-ascus" stage. The first half of March was marked by temperatures above the normal for that month. The snow rapidly disappeared, leaving the leaves in a moist condition for several days. Thermograph records show that between March 7 and 21 there was a total of 120 hours when the temperature taken among the leaves remained above 4° C. Temperatures above 10° C. were recorded for 42 hours during this period. Frequent examinations of perithecia in leaves which had been placed on the ground on September 20, 1926, showed that asci developed rapidly during this period. By March 14 about 2 per cent of the asci in the more advanced perithecia contained mature ascospores. Following March 21 there was a period when the temperatures among the leaves seldom went above 4° C. No discernible maturation of ascospores took place during this period, but practically one hundred per cent of the asci in the more advanced perithecia had reached approximately mature size by March 26.

RELATION OF MOISTURE TO THE DEVELOPMENT OF THE ASCIGEROUS STAGE

Several authors have contributed to our knowledge of the relation of moisture to the discharge of ascospores of *V. inaequalis*. Frey and Keitt (5) observed that discharge in the orchard occurred when the leaves were wet by rain. Wetting by dew was not found to give discharges of consequence. As was pointed out earlier in this paper, Howitt and Evans (6) report a closer relation of the average mean temperature of January, February, and March to the time of primary ascospore discharge than the amount of precipitation from April 1 to the time of primary discharge. Owing to the nature of their data they were not able closely to define the relationship of moisture to ascospore development. The importance of rain in the spring in hastening ascospore maturity has been pointed out by Keitt and Jones (8), who observed that drought retarded development and primary discharge. Under controlled conditions they state that . . . "discontinuous wetting led to more rapid maturation than continuous wetting," and . . . "at 16° C. and a relative humidity of 78 per cent . . . the maturation of perithecia was checked until the leaves were thoroughly wet."

The above-mentioned studies were concerned chiefly with the effects of moisture on the later stages of ascocarp development. So far as the pres-

ent writer is aware, no attempt has been made to determine the relation of this factor to the initiation and early development of perithecia. A knowledge of this sort would be instructive, inasmuch as the amount of precipitation during the autumn might possibly influence the time of ascospore maturity the following spring. Experiments have therefore been conducted in an effort to determine the relation of moisture to the entire process of perithecial production.

Methods

It has been difficult to devise methods whereby the moisture content of the leaves could be controlled within a narrow range. As a consequence, much of the work has been of a qualitative, rather than a quantitative, nature. Some experiments, however, have been conducted in which an attempt has been made to use leaves with various known moisture contents. Known weights of air-dry leaves were immersed in water for varying lengths of time. At the desired time the leaves were removed from the water, the water was removed from the surfaces of the leaves by placing them between filter papers, and a second weighing was made. The water content was calculated on the oven-dry basis.

Where no attempt was made to determine the moisture content of the leaves, the degree of moisture was regulated as follows: (*1*) air-dry; (*2*) moist enough to be fully pliable; and (*3*) wet. When initiation and early growth of perithecia were studied, leaves with the lesions scattered uniformly over the lower surfaces were taken from the tree and cut into pieces, and one piece from each leaf was placed in the desired moisture condition for from 10 to 12 days. The leaves were then cleared by the Peace (12) method, and the abundance and diameter of the perithecia were determined as described for the studies of the relation of temperature.

For the study of the effect of moisture on maturation of ascospores, leaves were taken from a supply in the orchard and cut into pieces. One piece from each leaf was placed in each of the moisture conditions mentioned above. At intervals the more advanced perithecia were picked from each fragment at each of the several moisture conditions and examined. The results were recorded as the percentage of the total number of asci observed which contained delimited ascospores (including those which contained mature ascospores), and the percentage containing mature ascospores.

Absorption and Retention of Water by Dead Apple Leaves

Before beginning the discussion of the results of the experiments with the fungus it seems desirable to consider the wetting properties of leaves.

It would be expected that there would be wide variations in the wetting properties of leaves, depending on their structure and the amount of disintegration they had undergone. The more or less constant type of leaves produced by each variety of apple would lead one to expect, however, that the wetting property of any one variety would fall within a certain range, providing the leaves had undergone the same amount of weathering.

It was noted during the progress of the work that the leaves of certain of the crabs, upon being immersed in water, would become pliable more quickly than those of the McIntosh variety. As was earlier noted, perithecia appear to mature earlier in the Virginia (crab) leaves than in the leaves of the McIntosh. It was thought that this difference might possibly be explained, in part, by the rate of water absorption and water retentivity possessed by the leaves. It might be expected that during autumns and springs of little rainfall the perithecia would develop most rapidly in those leaves which had the ability quickly to absorb and hold the moisture of each light rain, or possibly of dew. It could hardly be expected, on the other hand, that such leaf properties would be of any great value to the fungus during seasons of abundant rainfall, since even the leaves which were the most resistant to wetting would become practically saturated during prolonged moist periods. To obtain evidence on this question, leaves of the Virginia (crab) and McIntosh varieties which had been placed on the ground at the same time in the autumn were brought into the laboratory. Known weights of these leaves were placed, with noted surface uppermost, on screens, and subjected for 10 minutes to a uniform spray of water. The water was then removed from their surfaces by the method described earlier, and a second weighing was made. Averages of 14 such experiments showed that the crab leaves took up 57 per cent of their oven-dry weight in water, while the McIntosh leaves took up 42 per cent. Experiments to determine the rate of water loss from the leaves of these two varieties failed to show any constant differences. Experiments to determine whether there was any difference in the rate of growth of perithecia when the leaves of the two varieties were subjected to parallel wetting treatments are as yet incomplete and inconclusive. This phase of the investigation proved to be one which required much more work for conclusive results than was feasible in the time available.

Additional experiments indicate that leaves absorb increasing amounts of water until a point is reached where the methods used revealed no more absorption after an hour of immersion under normal air pressure. If the air of these leaves was then exhausted, cases were noted where 32 per cent more moisture was absorbed. On the other hand, leaves which had been wet in the orchard for several days were found to weigh the same after the air was exhausted as before.

Growth of the Fungus into the Interior of the Dead Leaf

Growth of the hyphae from the subcuticular stromatoid layer into the interior of leaves taken from the tree in the autumn did not take place when the leaves were kept air-dry in the laboratory for 20 days. When similar leaves were moistened, however, ramification into the lamina took place within three days.

That the moisture present in the green leaf was sufficient to permit penetration of the fungus was shown by placing such leaves in a petri-dish to prevent drying. Within three days the fungus was found in the interior of the leaf. In no case, however, was the fungus found to have penetrated the interior tissues of the leaf while it remained green, but it did so shortly after the leaf turned brown. It was noted that temperature was important in hastening browning of the leaves, those at the higher temperatures turning brown first.

Initiation and Early Growth of Perithecia

No initiation of perithecia was found in leaves which had been kept air-dry in the laboratory for one month. When leaves were moist enough to be fully pliable, however, perithecia were initiated readily. Moisture in excess of this did not seem materially to hasten initiation and early growth. Leaves were taken directly from the tree and divided into two lots. One lot was moist enough to be fully pliable, and the second was kept wet. After being kept for 12 days at 14° C., samples from each lot were cleared by the Peace (12) method. Measurements of 100 perithecia taken at random in each lot gave average diameters of 48 μ and 50 μ, respectively.

The data presented in table 3 indicate that drought retarded development of the perithecia in leaves which fell on October 7, 1924. On the other hand, although the rainfall following the September 23 collection was much less than that following the September 9 collection, yet the average daily increase in diameter of perithecia was practically the same in both cases. An examination of figure 4 shows that the rainfall following the September 9 collection was evenly distributed over the 15-day period. It is impossible to say whether dew might not have influenced the growth of the perithecia in the September 23 collection. No data have been obtained on this point, but the control experiments indicate that leaves may absorb moisture from the highly humid atmosphere of the Altmann apparatus, especially at the lower temperatures, and that perithecia may be initiated under these conditions in the absence of applications of liquid water. It can be conceived that the dews of certain types of autumn weather may be sufficient to permit initiation of perithecia.

Growth of Asci and Maturation of Ascospores

During dry periods of the spring months the perithecia seem to develop little if any. Rains followed by cloudy weather are conducive to rapid maturation of ascospores, providing the temperature is favorable. The conditions prevailing during an average spring would cause alternate wetting and drying of the leaves at intervals. In unusually wet springs, such as 1924, the leaves would remain wet for considerable periods. The question of whether protracted wet periods are favorable for maturation of ascospores is important, since early infections of the host are dependent upon comparatively long moist periods, and since, if ascospores were continually coming to maturity under these conditions, severe infections would result.

The experiment of discontinuous wetting reported by Keitt and Jones (8) was repeated with a slight modification. Leaves bearing the fungus in abundance were cut into pieces, and one piece from each leaf was placed in a petri-dish under each of the following moisture conditions: (*1*) wet for several minutes then placed in the dish, (*2*) wet for the same length of time as the first but the surface moisture removed between filter-papers before placing in the dish, (*3*) started wet (as No. 1) but allowed to become air-dry after two days, and thenceforth subjected to two-day periods of alternate wetting and drying. The results are presented in table 8. They are in general agreement with the results of Keitt and Jones in that the alternate wetting and drying led to more rapid maturation than continuous wetting. On the other hand, the leaves which were kept constantly "moist" contained perithecia which were at practically the same stage of development as those which had been alternately wet and dry. This does not necessarily mean that continuous moisture over a prolonged period is so favorable to normal development as discontinuous wetting. It has been found that, even at temperatures as low as 10° C., prolonged moistening of the leaves leads to aberrant development of the asci. Fixed and stained material from such leaves shows that there is often a development of vegetative tissue within the lumen of the perithecium, with the result that the asci are crowded to the top of the ascocarp. It would appear that the alternate wetting and drying as it occurs in nature leads to more normal development of the perithecia.

Experiments in which attempts were made to hold the moisture contents of the leaves at a certain known point have not been very satisfactory. As was stated earlier, if leaves are immersed in water for an hour or two, a point is reached when no more moisture will be immediately taken up upon further wetting, or it will be taken up so slowly as not to be detected with the method used. If, however, these leaves are exhausted of their air

under reduced pressure, more moisture will be absorbed, or if the leaves are left in water for a day or two the same thing will happen. While the experiments are not conclusive as to whether development of ascospores will go on in leaves which are entirely saturated, they indicate that a comparatively high moisture content is necessary for the most rapid maturation.

TABLE 8.—*Effect of different moisture contents of leaves on the maturation of ascospores of Venturia inaequalis, Madison, Wis., 1926*

Treatment of leaves	Stages of perithecial development after 12 days		
	Asci with delimited ascospores[a]	Asci with mature ascospores[a]	Discharge upon wetting the leaves
	Per cent	Per cent	
Wet constantly			
1	16	3	None
2	0	0	None
Moist constantly			
1	71	63	Fair
2	86	62	None
Alternately wet-dry			
1	90	63	Fair
2	80	30	Fair

[a] Percentage of total number of asci observed which contained delimited or mature spores. The more advanced perithecia were picked from the leaves for these examinations.

RELATION OF THE AGE OF THE LEAF TO THE PRODUCTION OF THE ASCIGEROUS STAGE

For two years the youngest terminal leaves have produced perithecia as early and in essentially the same abundance as the oldest terminal or spur leaves. The writer has found no evidence that the age of leaves affects their relation to the production of the perfect stage.

SUMMARY

1. The present work has been concerned primarily with the ascigerous stage of *Venturia inaequalis* (Cke.) Wint. in relation to some of the more important factors which influence its development. Special consideration has been given to the effect of these factors on the production of a timely and abundant ascosporic inoculum for the initiation of apple scab epidemics.

2. Penetration of the interior of the leaf by hyphae from the subcuticular stromatoid layer of the fungus and the production of perithecia take

place at any point beneath the indefinitely margined lesions, while these developments take place, in the main, only at the periphery of the more definitely margined lesions. The failure of the fungus to penetrate from the cells of the stromatoid layer near the center of the latter type of lesion seems to be correlated with the lack of protoplasmic contents in these cells. There seems to be an inverse relationship between the number of conidiophores produced from a given area of the stromatoid layer and the presence of protoplasm in the cells of this area.

3. Evidence was not obtained that hyphae of the fungus ramify the tissues of infected leaves extensively after leaf-fall and produce perithecia at points remote from lesions. Perithecia were rarely found more than one centimeter from a scab lesion.

4. Lesions which had been macroscopically invisible while the foliage was green were often noted after the leaves had become yellow. This may account, in part, for the conception that the hyphae of the scab fungus ramify the interior of the leaves extensively.

5. No indication has been found that the fungus penetrates the tissues of uninfected leaves on the ground and there produces perithecia.

6. The results of the studies reported above indicate that the quantity of perithecia produced in overwintered leaves bears a direct relationship to the type and abundance of lesions on the leaves.

7. Studies of the development of perithecia and descriptions of the grosser stages by which this development may be traced are reported.

8. Delay in the time of leaf-fall is accompanied by delay in the time the ascospores reach maturity the following spring. This delay of maturation of ascospores is not, however, commensurate with the delay in leaf-fall.

9. Meteorological factors, cultural practices and soil, the scab disease, and varietal differences of the apple appear to be important in inducing variability in the time of leaf-fall.

10. While perithecia developed in the leaves of all the apple varieties studied, they appeared to mature earlier in the leaves of some varieties than others.

11. Hyphae penetrated from the subcuticular stromatoid layer into the interior of the leaves at temperatures ranging from 4° to 28° C.

12. The optimum temperature for initiation and early growth of perithecia in oatmeal agar, agar made from dead apple leaves, and naturally infected dead leaves was near 13° C. Certain differences which were found when the fungus was grown on malt extract agar are discussed. On apple-peel and oatmeal agars short exposures to temperatures near 0° C. appeared to stimulate perithecial initiation, while this was not noted when the fungus was grown on apple-leaf agars. Limited data strongly suggest that perithecia may be initiated at temperatures below 0° C. These data are, however, not conclusive.

13. The optimum temperature for maturation of ascospores appears to be near 20° C. Temperatures of 24° C. and above are detrimental to normal ascospore development if the leaves are moist. No deleterious effect was observed, however, when air-dry leaves were kept at temperatures above 24° C. for eight days.

14. There appears to be a transition from a lower to a higher range of thermal requirement as the perithecia develop. This phenomenon is discussed in relation to the shortening of the period from leaf-fall to maturation of ascospores when leaf-fall is delayed.

15. Minor consideration was given to the absorption and retention of water by dead leaves of different varieties of apples and the possible relation of these two factors to the production of perithecia in the leaves.

16. Hyphae were not found to grow from the subcuticular stromatoid layer into the interior of air-dry dead leaves, but such growth took place readily when the leaves were moist.

17. Perithecia were initiated as readily in leaves which were moist enough to be fully pliable as in leaves which were wet.

18. Perithecia matured more rapidly in leaves which were alternately wet and dry than in leaves which were continuously wet. On the other hand, when the leaves were moist enough to be fully pliable, the perithecia matured as rapidly as those in leaves which were alternately wet and dry. However, continuous moisture led to certain abnormalities in perithecial development.

19. Young terminal leaves produced perithecia as readily and in apparently the same abundance as the older spur or terminal leaves.

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LITERATURE CITED

1. ADERHOLD, R. Die Fusicladien unserer Obstbäume. II. Tiel. Landw. Jahrb. **29**: 541–588. 1900.
2. CLINTON, G. P. Apple scab. Ill. Agr. Exp. Sta. Bul. 67: 109–156. 1901.
3. EWERT, R. Der Einfluss des Fusicladiums auf den Laubfall. Landw. Jahrb. (Ergänzungsband) **48**: 157. 1915.
4. FREY, C. N. The cytology and physiology of *Venturia inaequalis* (Cooke) Winter. Trans. Wis. Acad. Sci., Arts, and Letters **21**: 303–343. 1924.
5. ———— and G. W. KEITT. Studies of spore dissemination of *Venturia inaequalis* (Cke.) Wint. in relation to seasonal development of apple scab. Jour. Agr. Res. **30**: 529–540. 1925.
6. HOWITT, J. E. and W. G. EVANS. Preliminary report of some observations in ascospore discharge and dispersal of conidia of *Venturia inaequalis* (Cooke) Winter. Phytopath. **16**: 559–563. 1926.

7. JONES, F. R. Is the *Venturia* of the Wisconsin wild crab, *Pyrus coronaria*, identical with the *Venturia inaequalis* on the cultivated apple? Unpublished thesis, Univ. of Wis. 1914.
8. KEITT, G. W. and LEON K. JONES. Studies of the epidemiology and control of apple scab. Wis. Agr. Exp. Sta. Res. Bul. 73. 1926.
9. ———— and E. E. WILSON. Studies of the development of the ascigerous stage of *Venturia inaequalis* in nature (Abst.). Phytopath. **16**: 77. 1926.
10. KILLIAN H. Über die Sexualität von *Venturia inaequalis* (Cooke) Ad. Ztschr. Bot. **9**: 353–398. 1917.
11. LAWRENCE, W. H. The apple scab in western Washington. Wash. Agr. Exp. Sta. Bul. 64. 1904.
12. PEACE, L. M. Notes upon the clearing and staining of leaves and stems. Plant World **13**: 93–96. 1910.
13. SCHNEIDERHAN, F. J. and F. D. FROMME. Apple scab and its control in Virginia. Va. Agr. Exp. Sta. Bul. 236. 1924.
14. VAUGHAN, R. E. A method for the differential staining of fungous and host cells. Ann. Mo. Bot. Gard. **1**: 241–242. 1914.
15. WALLACE, E. Scab disease of apples. N. Y. (Cornell) Agr. Exp. Sta. Bul. 335. 1913.
16. WILTSHIRE, S. P. Infection and immunity studies on the apple and pear scab fungi (*Venturia inaequalis* and *V. pirina*). Ann. Appl. Biol. **1**: 335–350. 1915.

EXPLANATION OF PLATES

PLATE V

Sections of perithecia of *Venturia inaequalis* in various stages of development in apple leaves.

A. Perithecial initial, showing origin from coiling of hypha (×382).

B. Formation of ascogonium from ascogonial initial which is at first straight but later becomes curved (×535).

C, D. Perithecia before trichogyne has been formed. Ascogonia one to several septate (×535).

E. Formation of trichogyne by elongation of one end of ascogonium which pushes through wall of perithecium (×535).

F. Perithecium with antheridium near it (×125).

G. Perithecium with long trichogyne (×535).

H. Ascogonium beginning to disappear. At this stage hyphae begin to appear in the lumen of the perithecium (×522).

I. Perithecium just before the appearance of the ascogenous hyphae. The remains of the ascogonium appear at the base of the perithecium and the remainder of the lumen is filled with hyphae (×352).

J. Somewhat later than I. A few ascogenous hyphae have appeared at the base of the perithecium (×618).

K. Appearance of asci. The ascogonium has entirely disappeared (×518).

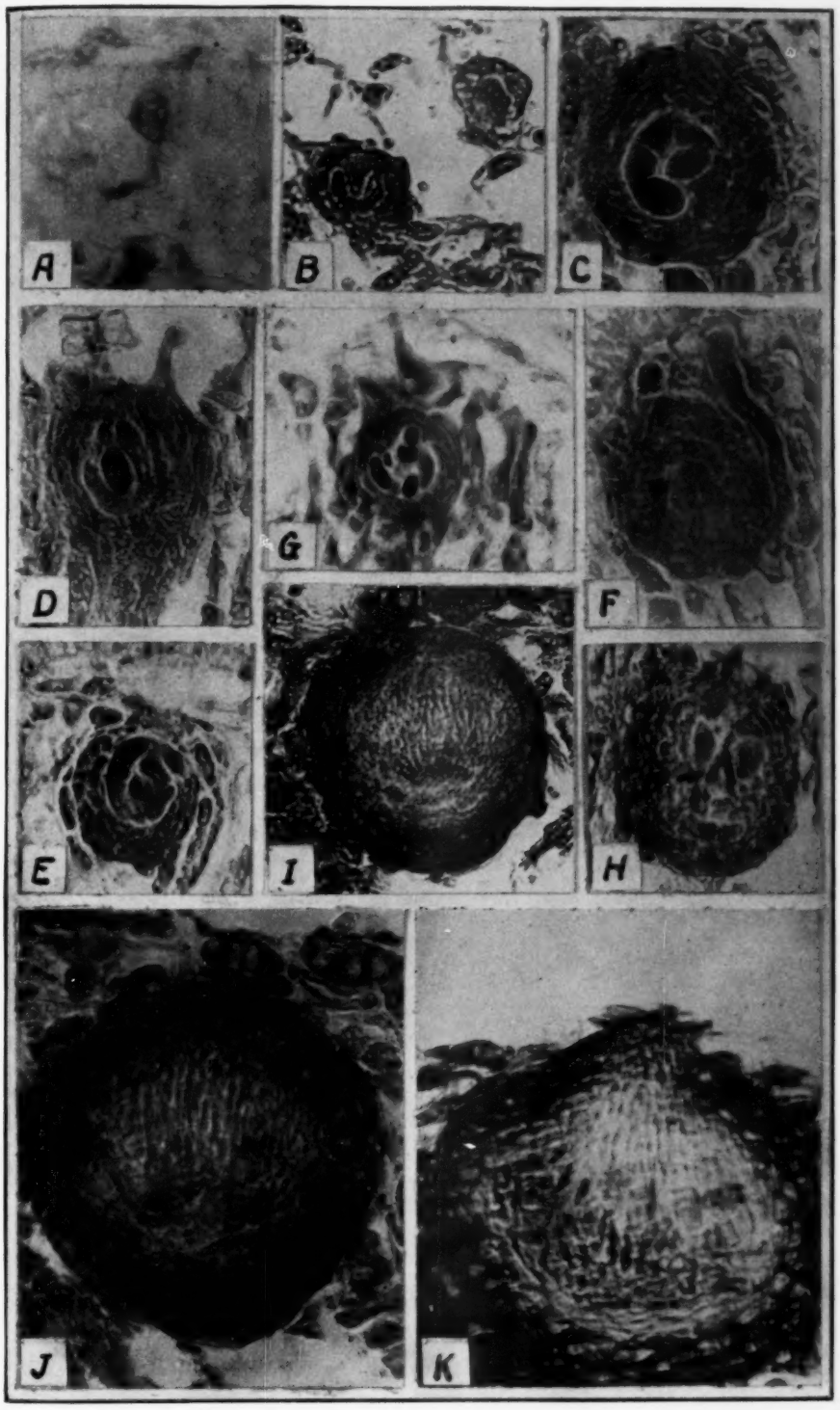
PLATE VI

Sections of perithecia of *V. inaequalis* showing later stages of development in apple leaves.

A. Asci about one-half mature size. Hyphae are still present in the lumen of the perithecium (×368).

B. Delimitation of ascospores (×368).

C. Mature ascospores. The hyphae have entirely disappeared from the lumen of the perithecium (×560).



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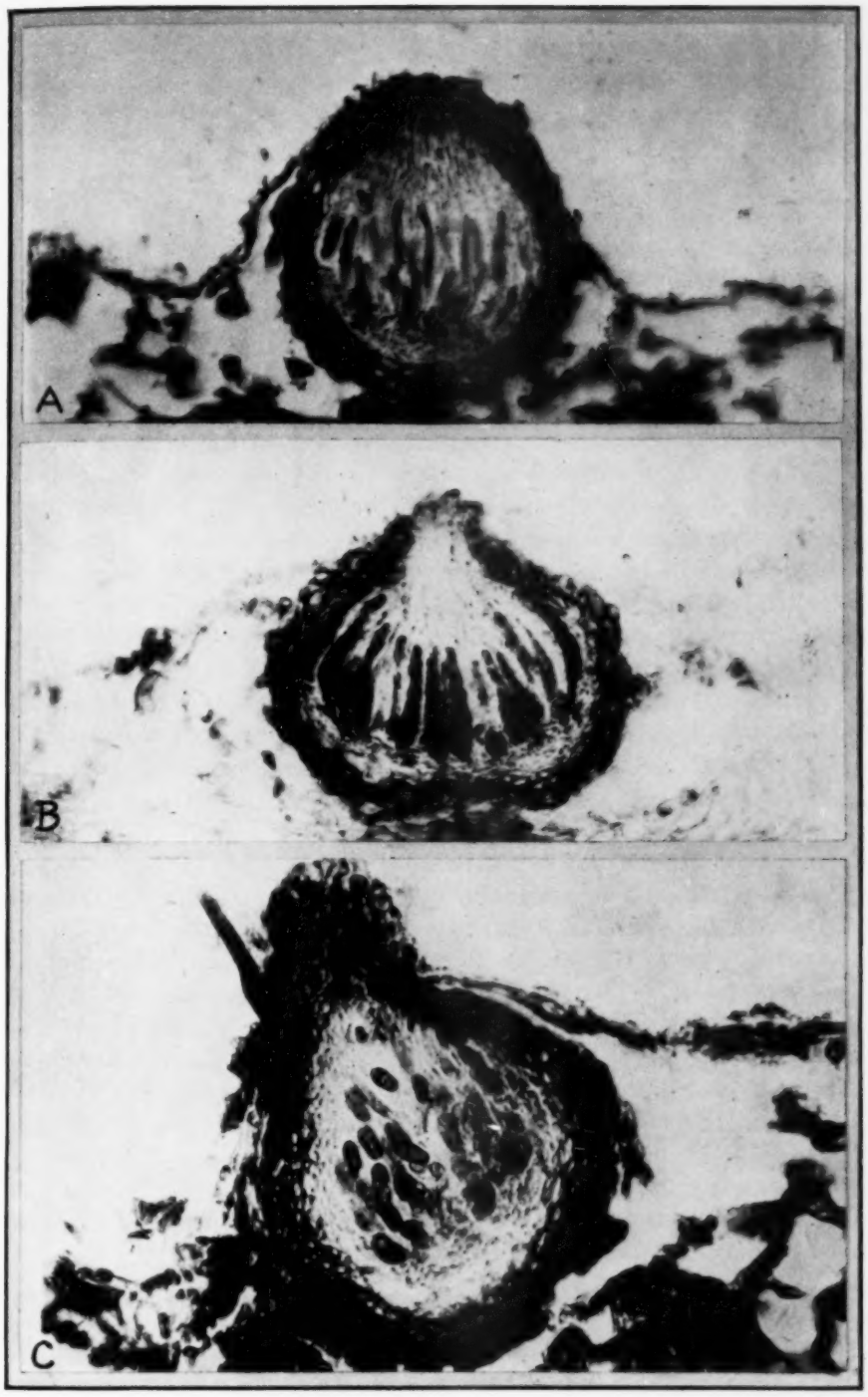

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A CONSIDERATION OF THE PATHOGENICITY OF THE COTTON-WILT FUNGUS, FUSARIUM VASINFECTUM[1]

H. R. Rosen

Attention was recently (12, 13) called to the fact that the growth of the cotton-wilt fungus, *Fusarium vasinfectum*, on a medium containing nitrate led to a reduction of this substance into nitrite. It has also been shown that nitrites in quantities comparable to the amount found in the nutrient media are extremely toxic to cotton.

Assuming these statements to be correct (the writer realizes that a great deal more quantitative work is necessary and that other factors may be involved in the use of nitrate), what would be the effect of adding nitrate to a soil which is poor in nutrients and in which the cotton-wilt fungus predominates? To answer this question, a series of soil experiments was undertaken involving the use of Ottawa silica sand and of a light sandy loam soil. The first, a whitish quartz material, was utilized because of its relative freedom from any active chemical ingredients, and the second to enable a direct comparison with soils which are frequently infested with the wilt-producing fungus.

Altogether the series consisted of 24 jars, mostly of a one-gallon capacity. With some variations, which do not concern us for the present, each treatment was triplicated and the results here given represent the averages of the various trials. The variety of cotton seed utilized was a strain of Trice that is very susceptible to wilt. A rather heavy inoculum was mixed thoroughly with the sand and soil, and a somewhat large amount of sodium nitrate was used, though not in sufficient quantities to produce injury. The amount added to each jar approximated about 1,000 pounds to the acre. Table 1 gives the percentages of germination obtained.

The outstanding result of these tests is the complete suppression of germination in all pots in which $NaNO_3$ existed in the presence of the fungus. (See Figs. 1 and 2.) This is true not only of the pure quartz tests, but also of the sandy loam ones, and it appeared in sterilized as well as in unsterilized soil.

The seeds, the same number in each jar, were planted at the same time, on July 1, immediately following the application of the inoculum. The latter, as previously noted, was used in relatively large quantities, the con-

[1] Research paper No. 82, Journal Series, University of Arkansas.

TABLE 1.—*The percentages of germination obtained with cotton seed grown in soils infested with Fusarium vasinfectum and fertilized with $NaNO_3$*

Type of substratum	Percentage of germination
White sand plus inoculum	80
White sand plus inoculum plus $NaNO_3$	0
White sand, no inoculum	73
White sand, no inoculum, plus $NaNO_3$	73
Sandy loam plus inoculum	57
Sandy loam plus inoculum plus $NaNO_3$	0
Sandy loam, no inoculum	64
Sandy loam, no inoculum, plus $NaNO_3$	60

tents of a 500 cc. Erlenmeyer flask for each jar. Within three days, germination had started in some of the jars, and within a week practically all germination had ended. Notes, however, were taken up to 21 days following planting. The work was conducted in a greenhouse where the temperature fluctuated, but the fluctuations in temperature were not so great perhaps as would be experienced in the same period out of doors. Roughly speaking, the temperature averaged about 28° C., varying from about 25

FIG. 1. Ottawa silica sand experiments. The two lower jars were heavily infested with *Fusarium vasinfectum*, the two upper were free from the fungus. The two jars at the right received nitrate of soda, while those at the left received none. Note the complete failure of germination in the infested pot which contained nitrate. Photographed two weeks after seeding.

to 30° C. The early afternoon temperature, depending on the brilliancy of the sunlight, was apt to be higher than that out of doors, while the night and morning temperatures were also somewhat higher. Owing to the fact that the period in which this experiment was conducted had a high proportion of cloudy days, extremes of heat were not experienced. As the local water supply was heavily chlorinated, distilled water had to be used for watering. No effort was made to run the tests more than three weeks, as the primary object was to obtain the data on germination, which were, by the way, not entirely unexpected.

The experiment clearly brings out the relationship of the cotton-wilt fungus to the germination of the seed, and the marked difference in percentage of germination due to the presence or absence of suitable quantities of nitrates in the soil. In other words, given the same kind and relatively the same quantity of fungus in the soil, cotton seed may or may not germinate, depending upon the chemicals present in the soil. These results may in part, at least, explain the frequent unevenness and lack of stand of cot-

FIG. 2. Sandy loam soil experiments. The jars are directly comparable to those shown in Fig. 1. Here also germination was completely inhibited in the infested jar containing nitrate of soda, the lower right one. Photographed the same time as Fig. 1.

ton, though it does not answer the question completely. It should be noted that the replanting of cotton two or three times in one season is not confined to seasons of floods; it is all too common in ordinary seasons. Also, the huge quantities of cotton seed planted, necessitating a large amount of labor in "chopping" or thinning, are required partly on account of the relatively high mortality of cotton seed under varying field conditions.

This is not the first time that attention has been called to the possible action of the cotton-wilt fungus in preventing germination. In a description of some field experiments (11) undertaken about four years ago, it was noted that certain fertilizer plots showed stands far superior to adjoining unfertilized plots, but that such superiority existed only where potash alone was used and was unnoticed where a complete fertilizer was present. The whole field, about one acre in size, had been heavily infested with pure cultures of the cotton-wilt fungus, the infestation being so great that "within a few days after planting and before the plants had appeared, a heavy fungous growth could be clearly seen across the field, marking the paths where the cultures had been applied." The explanations then offered for the better stands on the potash plots and for the poorer stands on plots with complete fertilizer were purely conjectural. The work here recorded offers a better basis for an explanation, and it is as follows.

In the presence of the fungus, those plots which had received a complete fertilizer had a lower percentage of germination because of the presence of the nitrate of soda (at the rate of 120 pounds to the acre); and it is quite conceivable that wherever the concentration of nitrate and its reduction product, nitrite, was great enough, lack of germination was encompassed. Of course, in addition to the toxic action of nitrite, there is still the possibility that under certain conditions potassium salts, by influencing the metabolism of the host plant, may act as preventives of reduced stands or of wilt development. The belief that wilt can be controlled by the use of potash is tenaciously held by some planters, although Orton's work (10) and that of Smith and Lewis (17) seem to disprove it. A great deal more work is required under various soil conditions before it can be entirely rejected.

The early work of Appel (1) and Schikorra (14) on Fusarium diseases of certain legumes is worthy of attention in connection with the studies here recorded. Appel found that if healthy seed of field peas are inoculated with a spore suspension of *Fusarium vasinfectum* var. *pisi* van Hall, or if such seed are planted in infested fields, germination is inhibited. If seedlings develop, they become either immediate victims of the fungus or wilt later on. He finds the same thing true for the Fusarium disease of Lupines; but in *Vicia faba*, on the other hand, attacks by *Fusarium* are

limited in the seedling stage to parts of the cotyledons, and it is only in later stages of the development of the plant that the disease takes hold by means of the vascular bundles. Again, Appel notes that weak plants of *Vicia faba* and of *Lupinus angustifolius* are subject to a regular foot-rot, "Fusskrankheit," which kills the plant by means of the death of the lower part of the stem. The writer finds the same thing true for *Fusarium vasinfectum* acting on cotton seedlings when a heavy inoculum is used in the field or in pots kept in the greenhouse. Other factors such as temperature, moisture, and type of substratum also play an important rôle in the development of damping-off or foot-rot, caused by *Fusarium vasinfectum*. Elliott (5) has called attention to the marked influence exerted by the medium used for growing the cotton-wilt fungus in influencing the development of other organisms which may attack the cotton seedlings and produce damping-off. Cornmeal or cottonseed meal, when incorporated into the soil even in small quantities, "made so favorable a medium for fungous and bacterial growth that plants in both the control and inoculated pots were killed by almost any organism which chanced to infect the meal." Now *Rhizoctonia* in particular is often to be found causing damping-off even in sterilized soil, for, unless extreme precautions are taken, it takes but a relatively short exposure to ordinary greenhouse conditions for it and other soil inhabitants to invade the sterilized soil. Parenthetically it may be noted that sterilizing the soil by heat or by chemicals is apt to change the biological, chemical, and physical qualities of the soil to such an extent that comparisons with field behaviors of the same soil, and of any parasite associated with it, must be made with considerable caution. The point is, that while Elliott is doubtless correct in regard to the possibilities of various fungi and bacteria, other than the cotton-wilt pathogen, causing damping-off, nevertheless, *Fusarium vasinfectum* can also produce a cortical root-rot or a rot of the subterranean portions of stems, and this may manifest itself as a typical damping-off. Indeed, in the same publication he notes that, in some cases, plants growing in soil artificially infested with *Fusarium vasinfectum* died without showing positive indications of wilt but merely rotted off at the soil line, and very recently Fahmy (6) described a girdling of the hypocotyl of cotton seedlings grown in soil heavily infested with the wilt-producing organism. Further than this, it may be stated that the type and quantity of inoculum as well as the kind of soil has a very marked influence on the severity of infection and on the type of infection. More will be said of this in a later publication.

THE RELATIONSHIP OF FUSARIUM VASINFECTUM TO CORTICAL ROOT-ROT

It has been a common observation of various investigators that the cotton-wilt fungus in artificial infection experiments often fails to produce a

true wilting. Erwin F. Smith (16) noted it, and in his usual painstaking way undertook large numbers of artificial infections, but while he was successful in producing the Fusarium wilt of watermelons, he met reverses with the cotton pathogen. He says, "All of the cotton plant inoculations have failed. These also were soil inoculations, and were performed on many small plants, using the cotton fungus, the cowpea fungus . . . , and the melon fungus. The experiments were repeated in different years and were continued in some cases for a long time with both sea island and upland cotton." Even Atkinson (2), who was the first to discover and name the causal agent, had difficulty in inducing healthy plants to wilt. He tells of having isolated the fungus in pure culture and attempting artificial infections and says, "The *Fusarium* was considered not to be a sufficiently aggressive parasite to be able to make its way into the ducts of the circulatory system unaided. Having found that the damping-off fungus could disease the stems of the young cotton, and that many plants even when the ulcer reached the circulatory system recovered from the effects of this external injury, it was suggested that possibly this fungus could open the way for the entrance of the *Fusarium*." He then inoculated some young plants with the damping-off fungus (probably *Rhizoctonia*, judging from his description of the mycelium), and when some of these had contracted the disease so that "an opening was made in as far as the vascular tissue, a portion of the earth was removed and pure cultures of the *Fusarium* were placed directly against the diseased portions of the plants. . . . One plant about 12 inches high died exhibiting signs of the disease in one leaf," and he found by microscopic examination that the *Fusarium* occupied the ducts near the ground. In another single case he succeeded in producing wilt by placing stems of diseased plants near plants suffering from attacks of damping-off, and he remarks, "The diseased condition of the ducts (in plants that show damping-off) affords an opportunity for the fungus to gain a foothold." Orton (9), in 1900, was the first to produce artificial infections in a fairly adequate number of plants, and his views concerning the aggressiveness of the parasite, as then expressed, are diametrically opposite to those held by Atkinson. He says, "It is not believed by the writer . . . that the assistance of the root nematodes or any fungus is necessary to allow the wilt fungus to gain entrance to the roots of cotton. . . . The indications are that the fungus is a sufficiently aggressive parasite to make its way unaided into the vascular system of the plant whenever the plant is liable to infection." He obtained 14 successful infections out of 24 plants inoculated with pure cultures. Of these 14, 7 showed the typical wilt form of the disease, while all the check plants remained healthy. "That a larger proportion of the inoculations did not succeed is believed to be due to the

small amount of fungus used and to the natural resistance of the plants." Smith's failure to obtain infections he believes was due either to the slow growth of the plants or to their natural resistance.

It is a very difficult thing to believe that all of Smith's failures and those of almost every investigator who has dealt with this disease are due to the causes assigned by Orton, although, as will be shown later, natural resistance is possessed by most cotton varieties. Smith succeeded in producing infections with the watermelon *Fusarium*, and it is quite probable that the amounts of inoculum he used with this were comparable to the amounts he applied to cotton inasmuch as the experiments on these diseases were conducted at the same time. Again, Smith used a number of varieties of cotton and failed with all. The present writer has conducted relatively large numbers of experiments over a period of about seven years, involving field and greenhouse tests, using at times over 50 different varieties and strains, many of them extremely susceptible under certain field conditions; and with very few exceptions the typical field forms of wilt were not obtained. Some of these experiments will be detailed below. Elliott (5) likewise failed at times to obtain typical infections and attributed his failure to a variation in the pathogenicity of various strains of the cotton-wilt fungus. Very recently Singh (15), in India, made a careful study of a number of strains of *Fusarium* isolated from cotton plants naturally infected in the field and from various types of wilt-infested soils, and he was unable to obtain typical wilt by means of artificial infections. His conclusion with reference to the few cases where he obtained wilt is that the fungus "is not a virulent parasite on cotton plants, since it was found in plants, both in the inoculated and in the uninoculated series, only when the plants were attacked by *Rhizoctonia*." Of course the meaning of the term "virulent parasite" is not fixed and may be used variously. If Singh means that this organism cannot attack and produce disturbances of varying degrees of severity, then his statement is of doubtful significance. But if he means that *Fusarium vasinfectum* can only produce wilt under restricted conditions and that it is a wound parasite, requiring a nidus of dying or dead host cells before it can injure the living tissues, then the writer is in full agreement with him, having presented this view in a previous publication (13). Butler's (3) recent publication again calls attention to the fact that artificial inoculations are not readily obtainable with the cotton-wilt parasite; he believes that the organism is capable of definite pathogenic action only under certain conditions. These, he further believes, have not yet been elucidated and are probably not connected with the composition of the soil, as Dastur (4) has concluded. The latter has proposed the idea that wilting is related to the presence of iron and aluminum compounds in the soil, inasmuch as he was unable to produce wilt by artificial infections with the fungus which

had been associated with wilted plants. On the other hand, Fahmy (6), perhaps unintentionally, gives the impression that wilt may be produced at will by artificial inoculations; at least nothing is said about any failures to produce wilt artificially in susceptible varieties.

By what means does the organism penetrate and what are the first signs of the disease it produces? In the pot experiments recorded in the first part of this paper an excellent opportunity was afforded to study the invasions of the parasite, particularly in those jars where pure, quartz sand was used. This material does not stain the roots as ordinary soil does, and any lesion, even though it be small, is apt to appear conspicuous against the whitish background of the uninfected parts. In the jars which had not received nitrate and in which the fungus was incorporated, every plant, when carefully removed, showed a very large number of brownish lesions of varying sizes on the roots, while control plants in uninfested jars showed very few or none at all. A relatively large number of lesions, when examined under the microscope, showed the presence of the fungus, in some instances with the typical microconidia of *Fusarium vasinfectum*. In other cases the fungus was not observed, and the chances are that it had either perished or was obscured by the entrance of secondary invaders. Occasionally bacteria and other fungi were seen in such lesions. When surface sterilized and plated out on potato dextrose agar, the fungus was isolated in about 25 per cent of the cases, while the remainder were sterile or developed various types of microorganisms, often producing both bacterial and fungous growth. This, of course, is to be expected under the conditions prevailing about a root system. A careful histological study has not been made, but there was fair indication in about 50 per cent of the cases observed of a suberization process going on; and the probabilities are that, if the material had been embedded in paraffin and carefully sectioned and stained, the production of corky layers would have been detected in a greater percentage of the lesions.

One of the most significant findings with reference to this discussion was the frequency of lesions at the collar of roots, that is, in those natural wounds made by the passage of extruded, secondary roots. In very few instances were discolorations observed which did not center around wounded areas, and even in these instances it was difficult to tell with certainty if wounding of some sort had not occurred prior to the advent of the fungus. The general view presented by an infected root system under the conditions of these experiments was that of the action of a wound parasite, which in a large number of instances was barely able to gain a foothold on the host and produce more or less localized, superficial, cortical discolorations.

In addition to the large numbers of small cortical lesions, another effect noted was the lack of elongated feeding rootlets, particularly in the lower

parts of the system. The result was that the root complex usually consisted of one elongated tap root with a mat of short, feeding roots, bunched more or less together at the upper part of the root. It was quite obvious that the plant had lost a large number of the finer roots and that the bunching represented successive efforts of the plant to replace the roots that were being destroyed. As the control plants were under the same environmental conditions, other than not being in the presence of the fungus, there is very good reason to suppose that the symptoms here described are directly or indirectly due to the action of the introduced parasite. In addition to this evidence, the fungus has been located in a number of such decayed roots by microscopic observations and by pure culture isolations. This effect on the roots has, with very few exceptions, been entirely overlooked by students of this disease and by textbook writers, although Orton (9) called attention to it in 1900. He noted that tufts of roots grew from points of infections and that "several short roots would start from a place which would normally have produced one longer branch." Many of these smaller roots, he found, were killed by the fungus, and he considers the stunted top growth of such plants as due to the action on the roots. He found such root tufts both in artificial infections in the laboratory and on plants growing under natural field conditions. With reference to the latter, it is of considerable interest to note that these were observed in fields where Orton apparently did not find any typical wilted plants. For, he says, the presence of such malformations "on the roots demonstrates the presence of the wilt fungus in the soil, even when the amount is so small that no harm is visible aside from the reduced growth of the plants."

The writer's studies up to the present are not sufficient to indicate where and how infections started on these rootlets. Very frequently the discoloration and rotting is to be observed at or near the tip, and there is some reason to believe that the infections started when the roots were in the incipient stage, shortly after, or at the time when they broke through the overlying cortical tissue of the mother root. Inasmuch as the region around the opening made on the mother root by the passage of the rootlet is often infected, as previously described, it is quite possible that the fungus may have passed along from the wounded tissue of the mother root into the developing rootlet. There is also the possibility that the region of the root tip with its dead and dying cap cells offers abundant opportunity for the fungus to gain a foothold there, whence it spreads to the adjoining healthy tissue. It is important to note that, in spite of such numerous injuries produced by the fungus, it usually does not penetrate sufficiently into the vascular system to produce wilt.

THE ARTIFICIAL PRODUCTION OF WILT BY FUSARIUM VASINFECTUM

It has already been fully recorded that the production of a true wilt, comparable to that observed under natural conditions, is frequently unobtainable in artificial infections. By natural wilting in this disease is meant the loss of turgescence of foliar tissues and of succulent portions of stems, resulting in a drooping, withering, discoloration, and premature death of the plant. It should be distinguished from those symptoms induced by the same parasite resulting in various forms of root injury, described previously, which are more or less localized and which may result in a stunting of the whole plant or some of its parts. Likewise, it is to be distinguished from those forms of the disease in which no true wilting is involved, and in which the plant continues to live for relatively long periods, often up to the first killing frost, and also in which the disease may manifest itself as a more or less limited, often narrow, thread-like discoloration of parts of the vascular system. The latter is frequently obtainable in artificial infections when very little or no true wilting is to be had, and it not infrequently happens that the platings of portions bearing the discolored tissues remain sterile. Most of these internal discolorations do not appear until the plants are quite advanced in age, after a period of 60 to 90 days, and at the end of this time plants grown under ordinary greenhouse conditions are apt to be so weak and debilitated that one hesitates to ascribe any marked degree of virulence on the part of the fungus or on the degree of tolerance possessed by the host plants.

It may be worth while to describe some of the attempts at artificial infections in field and greenhouse experiments, undertaken primarily to test the degree of resistance to wilt by different varieties of cotton. A field approximately one acre in size, consisting of a rather heavy clay or silt loam soil, near Fayetteville, Arkansas, was heavily infested with pure cultures of a *Fusarium* which had been isolated from a badly wilted cotton plant. It is the field which has previously been cited (11) as showing a large amount of seedling damping-off and lack of germination, both of which have been ascribed to the fungous inoculum. The varieties used were Webber Deltatype, Express, Pedigree Register, commercial Acala Number 5, and Dix-Afifi. Of these, Dix-Afifi, and Express to some extent, are known to be resistant to wilt. The others, on the other hand, have been found to be susceptible to wilt under various conditions in Arkansas. Of the hundreds of plants used, only 18 developed a true wilt: 9 of the Webber Deltatype, 8 of the Express, and 1 of the Dix-Afifi. The wilting was noted in the period between June 12 and June 26, when the plants were about six to eight inches high, and the appearance of the vascular systems, as well as the isolations from them, left no doubt as to the type of disease. On the

other hand, all of the remaining plants showed no recognizable symptoms until late in the growing season, in September. At about picking time, when the plants were pulled up and their interiors examined, approximately 25 per cent showed linear discolorations of the bundles, involving all of the varieties. But, so far as yield is concerned, no appreciable difference could be detected between plants without interior discolorations and those with discolorations. The appearance of the plants was such that it was considered worthless to obtain data concerning yields of infected and uninfected plants. As far as gathering data concerning varietal resistance and susceptibility went, the experiment was at that time considered quite a failure. It is quite interesting to note in this connection that, while the cotton failed to wilt in this artificial soil infestation, there was almost 100 per cent wilt in susceptible varieties of tomato in a field not more than 150 yards away from the cotton field, also artificially infested with the wilt-producing organism, *F. lycopersici*.

Since the experiment detailed above was conducted outside of the main cotton-growing section of the state, it was thought advisable to utilize another field, within the cotton area. In 1923, an acre field near Van Buren was obtained which was so badly infested with wilt that about three-fourths of the plants were counted as wilted in 1922. The soil varied from almost a pure river sand to a sandy loam, and in years of high water the Arkansas River, which flows close by, covers a considerable part of the field. In the fall of 1922 it had been planted to winter oats, and when it was obtained in May of the following year the oats were turned under without being cut or harvested. In order to insure thorough soil infestation, the cotton seeds were dipped in a very heavy spore suspension, and in addition were given another spore dose, by means of a sprinkling can, as they lay in the furrow. The soil was then immediately raked over them. About 30 different varieties and seeds from 150 selections, representing disease-resistant plants, were grown. It would be burdening the record to list all of the varieties, but there were included commercial types of Acala, Rowden, Trice, Webber, and other varieties that are usually considered susceptible. Throughout the growing season only six wilted plants were observed, and this in spite of the fact that cotton wilt was quite common in the vicinity of this field. The experiment was repeated on the same field for three successive years, with approximately the same result. Each year a heavy inoculum was applied; in one case it consisted of a mixture of various strains, the fungus being applied as a spore suspension or as spores and mycelium growing on cotton stems. The amount of inoculum used in any one year was several times as great as that necessary to insure thorough soil infestation with the tomato wilt *Fusarium*. In 1924, nine wilted plants appeared, and in 1925 only six

plants wilted. Obviously such numbers are of little or no value in indicating resistant and susceptible varieties.

In 1925, in spite of three years of artificial infestations, the yield of cotton was more than a bale to the acre. It has been reported by a number of investigators that the cotton wilt fungus is capable of living in the soil for a number of years in the absence of a suitable host. If we assume that the cultures used for inoculum were non-pathogenic, it would still be necessary to explain the absence of wilt in a field which had been so thoroughly infested less than one year previously. Likewise, it does not seem proper to attribute this failure to lack of proper temperature, either soil or air, for, as previously indicated, adjoining fields in the section of the state around Van Buren showed various amounts of wilt in all three of the years, and large numbers of successful artificial infections have been obtained with *F. lycopersici* on tomato in the field and greenhouse, with the production of typical wilt, when very little or no wilt was obtained with the cotton pathogen, tried at the same time. Furthermore, Jones, Johnson, and Dickson (7) have concluded that all agricultural soils "tend to approximate the same temperature under the same meteorological conditions." It therefore seems reasonable to assume from these tests that cotton wilt may be more conditioned by the chemical, physical, and biological soil factors than are certain other Fusarium diseases, such as cabbage yellows, flax wilt, and tomato wilt. It will be recalled that tomato plants are like cotton plants in that they require rather high temperature for good growth. Is it possible that the growth of the oat crop and its use as a manure, in the interval between the successive crops of cotton, may have so changed the soil conditions as to render the plants more or less immune to wilt? More will be said of this in a future publication.

The artificial production of wilt in the greenhouse has sometimes been accomplished, but mostly the tests have been failures. There is evidence that part of these failures was due to the lack of virulence of some of the strains utilized for inoculum (See Figs. 3 and 4); and there is always the possibility of attenuating the parasitic habit of any microorganism which is grown on artificial culture media. On the other hand, the history of the parasite, and the length of time it may exist in the soil under field conditions, do not warrant the assumption that it loses its parasitic habits readily. Again, it seems too much to assume that such able investigators as Erwin F. Smith, G. F. Atkinson, and others did not have the true parasite or that their work was not otherwise carefully done. As a further check on the possibility that the true parasite was not at hand or that it became non-infectious after its isolation, soil from various parts of the State was obtained in which cotton had wilted badly, about eight weeks previously,

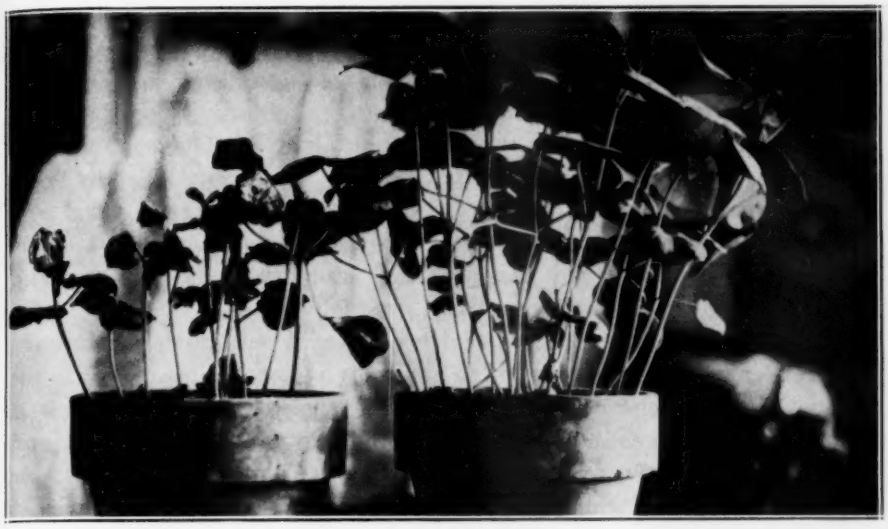

FIG. 3. A comparison between the pathogenicity of a strain of *Fusarium vasinfectum* isolated from a wilted cotton plant obtained in Arkansas (at the left) and one obtained from a wilted plant grown in Louisiana (at the right). The same number of seeds were sown in each pot, and the pots were otherwise under comparable conditions. The Arkansas strain had wilted a large proportion of the plants when the Louisiana strain had produced very little wilt.

under natural field conditions. Sufficient soil was obtained to enable the growing of several hundred plants. The greenhouse temperature under which they were grown was kept fairly well regulated, at a temperature of around 25° to 28° C., and although considerable seedling damping-off developed, there was very little true wilt. The plants were kept growing for about 40 days and then discarded. Less than 10 per cent had developed wilt, in spite of the fact that the seed represented a strain of Trice which was very susceptible to wilt under some field conditions.

If *Fusarium vasinfectum* were capable of wilting plants as readily as *F. lycopersici*, where would the American cotton industry be to-day? Cotton for a long time has been grown far more extensively and continuously in the South than tomatoes; and yet the cotton growers, taken as a whole, have paid next to no attention to the necessity of using wilt-resistant varieties, in spite of the fact that cotton wilt has been known in Arkansas, and in the South as a whole, for at least 40 or 50 years. Looking at it from another angle, if cotton wilt were always as destructive as the field photographs presented by various investigators show it to be, cotton growing by this time would have become a lost art. These photographs indicate what may happen in some fields, but they certainly do not indicate the average

condition in wilt-infested cotton fields. One is forced to conclude that this fungus is far more limited in its wilt-producing properties by certain factors than are other Fusaria. These factors have not as yet been entirely elucidated, but the work thus far seems to indicate the following possibilities.

It should be borne in mind that the cotton plant is a perennial and has a very firm, rather woody root and stem system. It is easily killed by frost, and consequently does not live more than one season under the usual conditions prevailing in the cotton-growing sections of the United States. Only when the plant is young and succulent is it most liable to serious root and stem injury by various microorganisms, especially *F. vasinfectum*. Therefore, conditions which make for succulence or which perpetuate the juvenile stage render the plant more liable to attack by root parasites. That is the reason, the writer believes, for the greater prevalence of wilt in wet seasons. Conversely, given good growing conditions, with a proper amount and balance of nutrients, even though there may be abundant moisture, the plant, by developing firm, woody tissues, is able to ward off the attacks of various root invaders. In the absence of good soil, or when some agent is

FIG. 4. A comparison between the pathogenicity of a strain of *Fusarium vasinfectum* isolated from a wilted cotton plant obtained in Mississippi (at the right) and one obtained from a wilted plant grown in Texas (at the left). The same number of seeds were sown in each pot and the pots were otherwise under comparable conditions. The Mississippi strain had wilted a large proportion of the plants when the Texas strain had produced very little wilt. The strain from Arkansas was very comparable in this respect to the Mississippi one while the Louisiana and Texas strain were closely related from the pathogenic viewpoint. (See Fig. 3.)

present in good soil which disrupts the tissues or which prevents the formation of wood and cork, the plant is particularly liable to invasion by *Fusarium*.

Thus it is, the writer believes, that nematodes, wire-worms or other insects, Rhizoctonia lesions, water levels which tend to asphyxiate roots, and other factors which destroy or break down root tissues, or which inhibit normal root development, render the plant susceptible to wilt. The action of nematodes on roots is largely that of producing localized, hyperplastic overgrowths, consisting mostly of soft, parenchymatous tissue and a reduced amount of cork and wood. It seems reasonable to assume that this production of soft, succulent tissue offers excellent opportunity for the growth of the cotton-wilt parasite, and this appears to be the explanation for the prevalence of cotton wilt on nematode-infested soil. It is not intended to suggest that factors other than mechanical ones are not involved in the resistance and susceptibility of cotton to wilt. It impinges on the question of parasitism in general, about which very little is known. And, even in the production of cork or wood, which may act as physical barriers, there undoubtedly enters a number of factors, many of them chemical, which must be considered. If we look at the question from the standpoint of ideal host development, we may be justified in assuming that most varieties of cotton are resistant to wilt when conditions are proper for their normal development, and it must be added that this does not necessarily mean the production of very large tops or of many bolls. The standard of normality here involved is the production of a hardy root and stem system, and more specifically perhaps, the inherent property of readily producing suberized layers. It may well be, according to this theory, that in so-called susceptible varieties, suberization of roots under some conditions does not take place as readily as in resistant varieties.

What are the conditions in ordinary pot or jar experiments conducted in the greenhouse? Are the plants more succulent than under ordinary field conditions, or are they more woody? Of course everyone knows that under these conditions there is apt to be an overcrowding of plants and that more plants will be grown than the soil can well support. This becomes more and more evident as the plants grow larger. They appear thin, wiry, more or less spindling, and are anything but succulent. As far as greenhouse cotton is concerned, succulence is relatively rare, except in the early seedling stages; and when it is considered that pains are usually taken to sterilize the soil, thus killing any nematodes, wire-worms, and various microorganisms which are capable of injuring root tissues and permitting ready access of the cotton *Fusarium*, the frequent failures to obtain wilt artificially are not so surprising. While a great deal remains to be determined about

the conditions which favor succulence in cotton, moisture and nutrients which make for unbalanced metabolism must be considered. The use of a quick acting and readily available source of nitrogen such as nitrate of soda will tend toward succulence unless other soil conditions are proper for the development of firm, woody tissues. Aside from the fact that the fungus is capable of reducing nitrates to nitrites which may kill some of the roots and thus enable the fungus to gain access to the plant, any nitrate which is taken up by the plant and not reduced by the fungus will make it more vulnerable to the action of *Fusarium vasinfectum*. On the other hand, when the soil is in a good physical condition and contains all the elements, properly balanced, for the development of firm, vigorous tissues, then cotton wilt will be a negligible factor. Of course the presence of nematodes or other organisms, or adverse temperature or moisture, which destroy the natural resistance of cotton plants, will tend to vitiate the action of any soil, no matter how good it may otherwise be.

Several months after the completion of this paper and after it had been submitted for publication, Neal (8) presented an extensive article on cotton wilt, including in this a study of various soil nutrients in relation to wilt production. He found, among other things, that when cotton plants were grown in washed sand to which various nutrients had been added a very small percentage of plants was killed by wilt, even after an interval of six months following the application of the inoculum. However, while the number of plants actually wilted was small (only one plant is definitely noted as having died of wilt), yet quite a few showed vascular and lateral root discolorations. From the latter symptoms he concluded that the disease was obtained in all the pots but varied considerably in the different nutrients. The most striking results to the present writer are those pertaining to the pots which received iron and in which the vigor of the plants is noted as being fair or poor. In these the results seem more consistent than in the others, hence more valuable, and clearly show a much greater number of infected plants. In another series of tests he used various amounts and kinds of commercial fertilizers in galvanized zinc buckets, and out of 40 plants subjected to the fungus, 12 became infected, of which 3 had received no potash. From this he draws the conclusion that potash, when used in an 8–4–4 or 12–4–4 formula and at the rate of about 800 pounds per acre, "may have increased the resistance of the plants to infection by the fungus." The results of one year's work on commercial fertilizers at two field stations are also given, and, as Neal notes, are quite inconclusive. Neal's work, taken as a whole, adds further emphasis to the writer's conclusion that the growing condition of the plants, especially soil nutrients, has a great deal to do with the presence or absence of wilt on any par-

ticular field, although it should be noted that it is only one element, potassium, that Neal seems to consider as having an important rôle in this phenomenon.

CONCLUSION

As all the evidence gathered thus far clearly indicates that most varieties of cotton grown in the United States show more or less resistance to wilt when conditions are proper for their normal growth and development, it seems reasonable to conclude that the control of wilt may be obtained by improving the growing conditions. The same result may also be accomplished by the use of certain hardy, resistant varieties which are capable of withstanding unfavorable conditions. There is danger of over-emphasizing the importance of one method of control to the exclusion of the other. Thus, while we have had several outstanding resistant varieties for about 25 years, they have been very little used in Arkansas and in other States, particularly since the boll weevil has come in. Such varieties as Dillon, Dix-Afifi, Dixie Triumph, and Cook are relatively late in maturing under the conditions existing in this State. They also possess other undesirable features, such as smallness of boll and a rather low percentage of gin turn-out. There is always the danger, in breeding for a certain character, of letting in other qualities which may not be desirable. Indeed, there is a question concerning the breeding of wilt-resistant cotton which has not yet been answered, namely, is wilt resistance correlated with lateness of maturity? Certainly most, if not all, of the resistant varieties developed up to the present are more or less late. It is of course possible that breeders may be able to overcome this difficulty, but in the meantime it seems to the writer that the other method of controlling wilt is worthy of consideration.

Almost all southern investigators of cotton are agreed that one of the greatest problems concerning the profitable production of cotton is the maintenance of soil nutrients. The continuous cropping of cotton, and burning up of soil organic matter, as a direct result of hot, subequatorial latitudes have so depleted the cultivated soil that profitable production is often unobtainable, even when prices are relatively good. Most unfortunately, from one point of view, the southern farmer learned long ago that cotton responds very readily to commercial fertilizers. Consequently, when plant foods were added to the soil they have usually been in the form of quick acting, readily available, and hence easily used up, inorganic salts. Manure or a green manurial crop, such as a legume, is rarely used in the South, compared to the North. Rotation is seldom practiced and when a legume is grown it is usually for hay or pasture. Under such conditions it is quite understandable how a crop which is really not a "heavy feeder" becomes subject to such wide-spread maladies as "rust" and wilt. "Rust"

is undoubtedly a malnutritional disturbance, and the writer has here attempted to show that wilt is also intimately connected with faulty nutrition.

From what has been said concerning commercial fertilizers, it must not be concluded that these are not desirable or profitable. The point is that they are often not sufficient to maintain a good soil fertility, although they may give immediate returns. It is of course well known to practical growers that when organic matter is present in the soil the cotton plant is able to make better use of commercial fertilizers. Good farming from this viewpoint will undoubtedly help in controlling wilt in the United States.

As far as foreign countries are concerned, the writer is not in a position to offer suggestions concerning control measures, because in some of them, particularly Egypt and India, the wilt seems to be prevalent on rich soils, which is rarely true in the United States. He would suggest, however, a much more thorough study of edaphic and climatic factors, particularly soil moisture, in relation to wilt development, as offering a possibility for control measures. It is of course conceivable that countries so widely separated and with different climatic and soil conditions may have developed strains of *F. vasinfectum* which differ not only in morphology but also in pathogenicity, as is indicated by Butler's (3) and Fahmy's work (6). While this may be true, private reports from various Indian pathologists seem to indicate that there also the parasitism of *F. vasinfectum* is limited by growing conditions of the host.

SUMMARY

Previous experiments having indicated that the presence of nitrates in nutrient media leads *Fusarium vasinfectum* to produce substances that are toxic to cotton, a series of greenhouse soil experiments were undertaken and here recorded, involving the use of pure quartz sand and of sandy loam soil low in nutrients.

In the presence of heavy infestations of the fungus, those jars which received nitrate of soda showed complete inhibition of germination, while the two types of controls, jars with equivalent amounts of nitrate but without any cotton wilt fungus, and jars with the fungus but without nitrate, showed no hindrance in germination.

A review of the literature indicates that almost every investigator who has worked with this disease has had considerable difficulty in producing artificial infections, with the production of typical wilt. This has led to a study of fungal penetration and of early disease symptoms.

It is shown that the fungus is primarily a cortical rot-producer of roots and of lower stem parts, and that its chief means of entrance is through wounds. Frequently it is found producing a limited, more or less super-

ficial, rotting of tissue about the natural wounds induced by extruded, secondary roots.

The fungus is also found to cause the death of elongated feeding roots without penetrating deeply enough to cause wilt.

Various field and greenhouse experiments are described in which attempts were made to produce wilt in artificial infections, and it is concluded that this fungus is far more limited in its wilt-producing properties by certain soil factors than are other Fusaria.

Succulence and the perpetuation of the juvenile stage are in particular pointed out as conducive to wilt. Factors which make for such plant development, as excessive moisture, and improper or unbalanced nutrients, are considered to be necessary for the fungus to produce wilt.

Likewise, any other agents, such as nematodes, wire-worms, and Rhizoctonia lesions, which are capable of disrupting root tissues or hindering their normal development, are found to make Fusarium invasion possible.

It is concluded that most varieties of cotton, when grown under proper conditions, are resistant to wilt, and that all observations seem to indicate that good farming practice, involving the use of manure or a green manurial crop, materially helps to control wilt in the United States.

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LITERATURE CITED

1. APPEL, O. Allgemeine Betrachtung über den Zusammenhang und Verlauf der in I geschilderten Leguminosen-Krankheiten und die daraus abzuleitenden Bekämpfungsmöglichkeiten. Arb. Biol. Anst. Land. und Forstw. **5**: 184–187. 1907.
2. ATKINSON, G. F. Some diseases of cotton. Ala. Agr. Exp. Sta. Bul. 41. 1892.
3. BUTLER, E. J. The wilt disease of cotton and sesamum in India. Agr. Jour. India **21**: 268–273. 1926.
4. DASTUR, J. F. A preliminary account of the investigation of cotton wilt in central provinces and Berar. Agr. Jour. India **19**: 251–260. 1924.
5. ELLIOTT, J. A. Cotton-wilt, a seed-borne disease. Jour. Agr. Res. **23**: 387–394. 1923.
6. FAHMY, T. The Fusarium disease (wilt) of cotton and its control. Phytopath. **17**: 749–767. 1927.
7. JONES, L. R., J. JOHNSON, and J. G. DICKSON. Wisconsin studies upon the relation of soil temperature to plant disease. Wis. Agr. Exp. Sta. Res. Bul. 71. 1926.
8. NEAL, DAVID C. Cottonwilt: a pathological and physiological investigation. Ann. Mo. Bot. Gard. **14**: 359–424. 1927.
9. ORTON, W. A. The wilt disease of cotton and its control. U. S. Dept. Agr., Div. Veg. Phys. and Path. Bul. 27. 1900.
10. ————. Cotton wilt. U. S. Dept. Agr. Farmers' Bul. 333. 1908.
11. ROSEN, H. R. *Fusarium vasinfectum* and the damping off of cotton seedlings. Phytopath. **15**: 486–487. 1925.

12. ————————. Efforts to determine the means by which the cotton-wilt fungus, *Fusarium vasinfectum*, induces wilting. Jour. Agr. Res. 33: 1143–1162. 1926.
13. ————————. The control of cotton-wilt by the use of organic fertilizers. Science 65: 616–617. 1927.
14. SCHIKORRA, G. Fusarium-Krankheiten der Leguminosen. Arb. Biol. Anst. Land. und Forstw. 5: 157–183. 1907.
15. SINGH, J. A study of Fusaria common to cotton plants and cotton soils in the central provinces. Mem. Dept. Agr. India Bot. Ser. 14: 189–198. 1927.
16. SMITH, E. F. Wilt disease of cotton, watermelon, and cowpea (*Neocosmospora* nov. gen.). U. S. Dept. Agr., Div. Veg. Path. Bul. 17. 1899.
17. SMITH, R. I., and A. G. LEWIS. "Black root" disease of cotton. Ga. St. Bd. Ent. Bul. 22. 1906.

STRAWBERRY DWARF

A. G. PLAKIDAS

INTRODUCTION

The purpose of this brief paper is to give a pathological description of a rather serious disease of the strawberry plant, which is prevalent in the strawberry-growing district of Louisiana, together with some observational and experimental evidence concerning the nature of the disease. It is recognized that the experimental evidence presented is not final or conclusive, but it is believed that a progress report at this stage of the investigation is justified. It is hoped that this apparently undescribed disease will thus be brought to the attention of other workers, and, in this way, more data will be obtained on its nature and geographic distribution. The writer has not been able to identify this disease with any of the strawberry troubles which have been described in detail, or with any of the more or less obscure root-rots or other strawberry troubles of which mention has been made in annual reports of different experiment stations. The term "dwarf" is proposed as a name for the disease.

DESCRIPTION OF THE DISEASE

The most conspicuous symptom of the disease is the severe stunting or dwarfing of the entire plant, a feature which suggested the name "dwarf." The leaves become greatly reduced in size, and are strikingly deformed. The petioles are short, and the leaflets rather elongated in comparison to their width. The leaflets are decidedly unsymmetrical, usually crinkled, and the margins cup upward in the young leaves and usually downward in the older ones. In extreme cases, the leaflets are reduced to mere rudiments. The older diseased leaves are slightly greener in color and more shiny than the healthy leaves of corresponding ages. The petioles, the veins, and the underside of the young leaflets are often reddish purple in color, though this is not a constant characteristic. Often some of the leaves of a plant are purple, while the rest are green. The affected leaves, both young and old, are decidedly brittle, as compared with the normal ones. Affected plants occasionally become "blind"; *i.e.*, the main bud is killed. Blind plants usually die, but occasionally several adventitious buds may develop from the crown. The leaves developed from these secondary buds are small, and their petioles are usually long and spindling. In general, a dwarfed mother plant transmits the disease to all daughter plants arising

from it, though occasionally runner plants may be found which appear perfectly normal. As far as macroscopic symptoms are concerned, dwarf is distinctly a disease of the top part of the plant, the root systems appearing normal in every respect. In figure 1 is shown a very typical specimen of

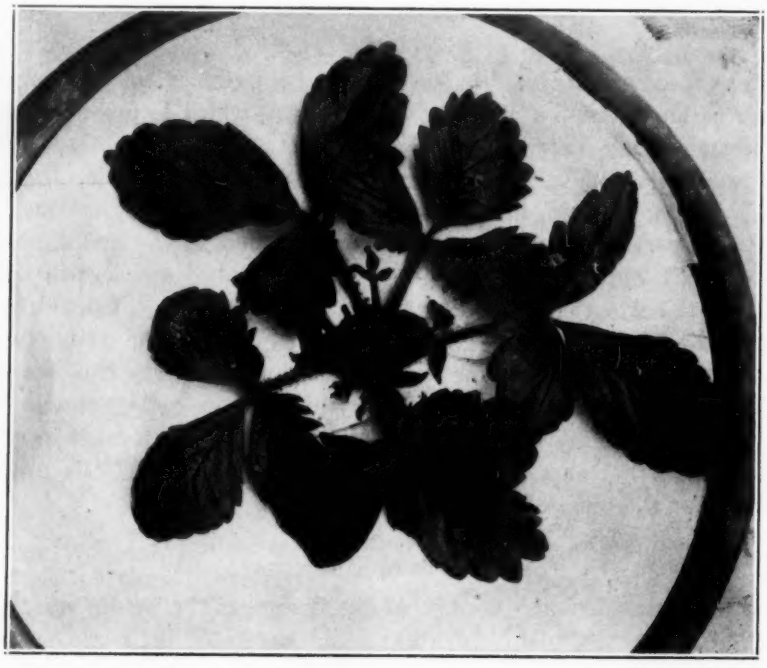

FIG. 1. Young Klondike plant showing typical dwarf symptoms. Note the crinkling of some of the older leaves and the extreme stunting and deforming of the younger ones. 0.7 natural size.

dwarf. The stunted growth of the entire plant, and the crinkled, deformed, and small leaves are clearly shown.

HISTORY AND DISTRIBUTION

It is not known where or when this disease originated. It has been recognized by the strawberry growers in Louisiana for a number of years, and it has been variously designated by such names as "wild plant," and "white bud." What suggested the name "white bud" is hard to see, for there is certainly no symptom of the disease which could suggest the term "white." The disease apparently occurs also in other parts of the South. It was found in a shipment of Klondike plants received by the Louisiana Experiment Station from a North Carolina nursery in the fall of 1927.

That the disease has been noticed in other parts of the South is shown by a letter received from Dr. Neil E. Stevens, of the Bureau of Plant Industry, in which he states in part: "There is a disease which we have been calling "dwarf" just as you do, common on Klondikes throughout the South, which seems to be transmitted from the mother plant to the offspring. . . . The one difficulty in working with it is that a good many plants which are stunted by drought or some such cause have a close resemblance to this dwarf condition. About a year ago we brought back from North Carolina a number of Klondike plants which we believed to be dwarf, kept them in the greenhouse during the winter, set them out under good cultural conditions in the spring, and now only something like one-third of the plants show any evidence of disease. This one-third, however, remains in the dwarf condition, and all of the daughter plants are infected. . . ."

The dwarf disease bears a certain resemblance to the strawberry yellows, reported from California,[1] especially with respect to the stunted growth of the plant and the reduction in size and crinkling of the leaves. In other respects the two diseases are apparently distinct. Chlorosis of the leaves, which is the chief characteristic of the yellows, is entirely absent in the dwarf. Furthermore, the dwarf is apparently peculiar to the Klondike variety in Louisiana, and this variety was found to be practically immune to the yellows in California.

IMPORTANCE

No accurate estimate of the damage caused by dwarf can be given at this time, but it is believed that the disease is one of the main causes of the marked reduction in yield of strawberry plantings in Louisiana during recent years. In fields in which the owners have recognized the disease and practiced systematic roguing, relatively few dwarfed plants are found. Incidentally, it may be stated here that those growers who are keen and careful enough to rogue and select their plants are the most successful in this section. In fields where little or no roguing has been practiced, 10 or 20 per cent of the plants may be found dwarfed. It is the unanimous opinion of all growers interviewed that dwarfed plants are practically worthless, for they produce very little fruit or none at all, and what is produced is of small size and has practically no market value.

ETIOLOGY

No pathogene of any kind has been found associated with the dwarf disease. Microscopic examinations of fresh and of fixed sections of leaves and

[1] Plakidas, A. G. Strawberry "yellows," a degeneration disease of the strawberry. Phytopath. **16**: 423–426. 1926.

roots of affected plants have failed to show the presence of any organism in the tissue. Furthermore, the disease cannot be considered as caused by any adverse soil conditions, since it is not limited to any particular soil type, or even to any particular spot in the field. Dwarfed plants are found scattered in the field, many growing side by side with healthy ones.

The general symptomatology of dwarf—the stunting of the plant, the crinkling, cupping and deforming of the leaves, and the fact that affected mother plants transmit the disease to their runner offsprings—suggests that dwarf may be a disease of the degeneration or virus type. The limited experimental evidence thus far obtained supports this view.

EXPERIMENTAL RESULTS

Twenty individuals of a strawberry aphis (probably *Aphis forbesi* Weed) taken from leaves of dwarf plants were placed on each of six young Klondike plants in 6-inch pots in the greenhouse. Each plant was covered by an insect-proof cage. Four other plants used as controls were likewise covered by insect-proof cages, but were not infested with aphids. The plants had been set in the pots ten days earlier, so that at the time the experiment was started only two or three leaves in each plant had unfolded. After the aphids had fed on the plants for ten days, they were killed by spraying the infested plants with a weak nicotine sulphate solution. The control plants were also sprayed with the same solution, to insure uniformity of treatment. The experiment was started on November 11, 1927.

Twenty-six days after inoculation, typical dwarf symptoms had developed in the young unfolding leaves of two of the six aphid-infested plants, and a third had doubtful symptoms. The rest of the inoculated plants, as well as all of the controls, were normal. On a subsequent examination, a week later, two additional plants in the inoculated lot had developed dwarf symptoms. One of the control plants had died from drought, while the remaining three were still healthy. In January, two months after the experiment was started, all six of the inoculated plants had developed the dwarf symptoms, while only one of the controls had developed the dwarf symptoms, the other two remaining normal.

The plants used in the experiment were of the lot of Klondikes obtained from North Carolina. In this lot a relatively high percentage of the plants was subsequently found to be infected with dwarf. Although the results obtained are suggestive, in view of the fact that in the inoculated lot every plant became diseased, they cannot be accepted as final, since it is not known how many of the plants used were originally free from infection. The fact that dwarf symptoms developed on one of the control plants indicated that some of the plants were originally infected even though they did not show

any dwarf symptoms at the time the experiment was started. On the other hand, it is highly improbable that all of the six inoculated plants were originally infected. Further experiments are now in progress, using a large number of plants, and it is hoped that more conclusive evidence as to the nature of dwarf may be obtained from them.

Additional evidence supporting the view that dwarf is a disease of the virus type was obtained from cytological studies. In the pericycle region of young roots of dwarf plants, cells are found which show marked signs of degeneration, and which contain from one to many black-staining (with the iron alum haematoxylin stain) amorphous bodies (Fig. 2). These cell

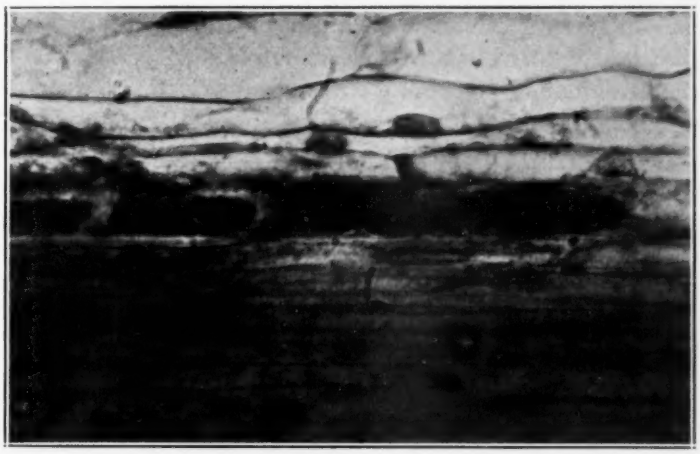

FIG. 2. Photomicrograph of a longitudinal section of a root tip of a dwarf plant showing degeneration of pericycle cells. Note the degenerated nuclei and the black-staining intracellular bodies. Iron-alum haematoxylin stain. Photographed with a Leitz 8× periplane eyepiece and with a Spencer fluorite oil imm. 2 mm. objective. ×580.

inclusions are strikingly similar in appearance and staining reaction to the "X" bodies found by the writer[2] in the pericycle cells of strawberry roots affected with the yellows disease, and by Rawlins[3] in the roots of sugar beets affected with curly-top. The degeneration of these cells is especially noticeable in the case of their nuclei. The nuclei of such cells are almost invariably devoid of nucleoli, and the nuclear membrane appears either to be practically empty, or to contain a small amount of granular, dark-staining material. What may be the nature of this cell degeneration and of the

[2] Plakidas, A. G. Strawberry xanthosis, a new insect-borne disease. Jour. Agr. Res. 35: 1057–1090. 1927.

[3] Rawlins, T. E. Cytology of root tips from sugar beets having the curly-top disease (Abst.). Phytopath. 16: 761. 1926.

intra-cellular bodies will not be discussed at this time. Further work is being done along this line. Suffice it to state that these abnormalities must have some relation to the disease, since they have not been found in healthy plants.

VARIETAL SUSCEPTIBILITY

No variety susceptibility tests have been carried out, but so far the disease has not been found on any variety except the Klondike. In Louisiana the Klondike is grown almost exclusively. The lot of plants from North Carolina contained, besides the Klondike, about 100 plants each of the varieties Missionary, Big Joe, Lady Thompson, and Hefflins Early; and, while nearly one-third of the Klondike plants proved to be infected, no case of dwarf was found in any of the other four varieties.

This is a preliminary report of a general study of strawberry diseases carried on in this laboratory under the direction of Dr. C. W. Edgerton.

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TRANSMISSION OF POTATO SPINDLE-TUBER BY GRASSHOPPERS (LOCUSTIDAE)[1]

R. W. Goss

It has been shown by Schultz and Folsom[2] that aphids are capable of transmitting the spindle-tuber disease. No reports have been found in the literature showing that other insects have ever been tested. Experiments in Nebraska have confirmed the published results of aphid transmission. However, a careful scrutiny of Nebraska fields during the past seven years has failed to reveal the presence of aphids in large enough numbers to account for all the transmission of spindle-tuber. In experimental plots designed for a study of the natural spread of this disease it was found in three successive years that the disease was transmitted from spindle-tuber to healthy plants in the absence of aphids. Tests were therefore made with other insects commonly found in potato fields.

In 1925, among other tests, 36 inoculations were made using grasshoppers as the transmitting agents. Eight of the inoculated plants became infected with spindle-tuber. The experiments were therefore repeated in 1926, and similar results were obtained.

METHODS

Essentially similar experimental methods were used both in 1925 and 1926. The experimental plot was located at the Scottsbluff substation, Mitchell, Nebr. The potatoes were grown under irrigation. All of the seed potatoes used had been indexed in the greenhouse and were planted in the field in tuber-units. All plants were cut back to a single shoot so that all the tubers in a hill were produced by one stalk.

Grasshoppers were obtained from non-solanaceous plants and were not collected from the vicinity of potato fields. The insects were first tested to determine whether they were already carrying the virus, by placing them on healthy plants either in cages covering the entire plant or in small cages used to cover a single branch. An uncaged plant in the same tuber-unit was used as a healthy control. After one to two days on this plant the grasshoppers were transferred to a caged spindle-tuber plant. They were allowed to feed on this plant two days and then transferred to a healthy plant,

[1] Published with the approval of the Director as Paper No. 46, Journal Series, Nebraska Agricultural Experiment Station.

Acknowledgment is due Mr. R. W. Samson, graduate assistant, who was in charge of the experimental field plots during the summers of 1925 and 1926 and who handled the detailed transmission tests in the field.

[2] Schultz, E. S., and Donald Folsom. Transmission, variation, and control of certain degeneration diseases of Irish potatoes. Jour. Agr. Res. 25: 43–117. 1923.

another uncaged plant of the same tuber-unit being used as a healthy control. In this manner a series of five plants was used for each inoculation.

In addition to these methods, however, an attempt was made to determine the effect of the number of grasshoppers used for an inoculation and the effect of repeated inoculations. The number of grasshoppers used varied from 1 to 10 in the various tests. Single inoculations were made by transferring the insects from a spindle-tuber plant to a healthy plant once, as described above. Double and triple inoculations were made by repeating this operation, the same insects being transferred back to the spindle-tuber plant and again returned to the healthy one. In all the above instances the grasshoppers were allowed to feed upon each plant for two days. The number of insects used in an inoculation was determined by counting only those which were alive and active during the inoculation period. At the end of the inoculation period the grasshoppers were killed with cyanide dust and mounted for identification.

All hills were dug by hand and bagged separately. Records were made of all symptoms appearing in the field and also of all the tuber symptoms. One tuber from each hill was indexed, *i.e.*, one seed piece was planted in the greenhouse under conditions favorable for the appearance of spindle-tuber symptoms. One tuber from a hill has proved sufficient for indexing when only one stalk a hill was allowed in the field, thus decreasing the possibility of partial infection of the hill. In all greenhouse indexing, the plants were grown until tubers were produced and records were made of the symptoms of both tops and tubers. In all inoculation series where positive or doubtful results were secured in the greenhouse, another seed piece from the same tuber as well as another tuber from the same hill was planted in the field the following summer.

If any of the healthy control plants showed symptoms of spindle-tuber in the field, or if the progeny developed symptoms, the entire series was discarded. Only two control plants out of the 140 used in 1925 were infected with spindle-tuber, and all the controls in 1926 remained healthy. The amount of uncontrolled transmission in the field, therefore, can be considered as negligible.

Most of the inoculations were made in late July and early August. The seed was planted about June 1 in both years and harvested about the middle of September.

RESULTS

Current season symptoms appeared on the tops in only a few instances. Symptoms were usually lacking in the tubers of inoculated plants; only 5 hills in 1926 produced typical spindle tubers out of 29 hills which proved to be infected when the tubers were planted.

By planting the progeny of inoculated plants and their controls it was found that 8 of the 36 plants inoculated with grasshoppers in 1925 had be-

come infected. In 1926 there were 29 plants infected from 64 inoculations. Considering the lack of any infection in the healthy controls in 1926, the percentage of infection (46.8 per cent) resulting from inoculations made with grasshoppers is very positive proof of the part these insects may play in transmitting the disease.

Table 1 shows the effect of the number of grasshoppers used for an inoculation and also the effect of repeated inoculations upon the percentage of infection. With increasing numbers and repeated inoculations the percentage of infection increased, until with triple inoculations infection was obtained in 65 per cent of the plants.

Six different species of *Melanoplus* were used in these tests.[3] The in-

TABLE 1.—*Effect of the number of grasshoppers and repeated inoculations with spindle-tuber on the number of infected potato plants in 1926*

Number of grasshoppers	Number of inoculations	Number of infected potato plants	Percentage of infection
	Single inoculations		
1	1	0	
2	1	0	
3	2	0	
5	3	1	
6	3	1	
8	1	0	
Total	11	2	18
	Double inoculations		
?	1	1	
2	4	1	
3	7	2	
4	5	3	
5	3	0	
6	8	5	
7	2	1	
8	2	1	
9	1	0	
Total	33	14	42
	Triple inoculations		
2	3	3	
3	6	2	
4	6	4	
5	3	2	
6	1	1	
7	1	1	
Total	20	13	65
Grand total	64	29	46.8

[3] Acknowledgment is made to the Entomology Department, Nebraska College of Agriculture, for assistance in the identification of the grasshoppers.

sects were not identified until after the experiments were completed and the insects killed and mounted. Only a few of the tests were found to have been made with unmixed species. *Melanoplus femur-rubrum*, *M. bivittatus*, *M. plumbeus*, and *M. augustipennis* were found to be able to transmit the disease in collections where an average of four out of six insects were identified from the collection. There is of course the possibility that the one or two grasshoppers which were not saved for identification may have been different species. Mixed collections of some of the above species with *M. parkardii* and *M. differentialis* were also successful in transmitting the disease. There is no reason for supposing that any one of these species would be more successful than the others in transmitting the disease in these experiments.

In the 1926 experiments it was found that, of the 77 plants upon which the grasshoppers were fed before being placed on the spindle-tuber plants, 4 became infected while the controls in the same units remained healthy. These same grasshoppers also transmitted the disease to the healthy plants upon which they were fed after feeding on spindle-tuber plants. The only apparent explanation is that these insects were carrying the virus when they were collected.

DISCUSSION

The successful transfer of the spindle-tuber virus to healthy plants by grasshoppers accounts for some of the previously unexplained spread of the disease, particularly in the irrigated districts of western Nebraska. In checking over previous years' results in experimental plots where the natural spread of the disease was being studied, it was found that the greatest amount of spread occurred in those plots where the records of insect infestation showed the greatest number of grasshoppers to be present.

The amount of positive evidence regarding the transmission of virus diseases of the potato by aphids and the few negative tests reported with other insects has tended to discourage attempts to transmit these diseases by many of the common potato insects. The part which aphids play in transmitting the spindle-tuber disease has probably been overestimated in some of the dry-land sections of the west where aphids are not common. Other experiments which have been conducted by the writer with other potato insects indicate that a number of them may be able to transmit the spindle-tuber disease. The experiments are being repeated with larger numbers.

A few attempts made to transmit mosaic with similar insects have given only negative results. In addition, in the plots being used for the study of the natural spread of potato virus diseases, it was found that both mild mosaic and rugose mosaic failed to spread under the same conditions that resulted in a considerable spread of spindle-tuber.

NEBRASKA AGRICULTURAL EXPERIMENT STATION,
LINCOLN, NEBRASKA.

COPPER SULPHATE AS A REMEDY FOR EXANTHEMA IN PRUNES, APPLES, PEARS, AND OLIVES

RALPH E. SMITH AND HAROLD E. THOMAS

The disease called "exanthema" or "dieback" in citrus trees has received considerable attention both in Florida and California. It is a trouble marked by very characteristic symptoms, as specific as those of any plant disease, parasitic or "physiological." So far as we are aware, exanthema has never been described on any but citrus trees, particularly the orange and grape fruit. For a long time, however, it has been known that in Cali-

FIG. 1. Four-year-old French prune trees with exanthema.

fornia a similar disease is not uncommon on fruit trees of various species, usually in spots of shallow soil where drainage is poor and the soil moisture varies between extremes of saturation and drouth. Defects of this sort are not obvious in every instance, however.

The recently discovered fact that application of copper sulphate to the soil has a strikingly remedial effect in some of these cases, as has long been known to be true with citrus exanthema, adds weight to the conclusion that the diseases are related and revives the interesting question as to the actual cause of this phenomenon.[1] A few cases may be described.

EXANTHEMA IN FRENCH PRUNES

A typical case occurred in a young orchard of French prune trees (*Prunus domestica*), planted on supposedly good soil which had originally supported a particularly heavy growth of the coast redwood or "big tree" of California (*Sequoia sempervirens* (Lamb.) Endl.) and other native trees (Fig. 1). This was in a region of heavy winter rainfall (50–100 inches) with no rain in summer (May–November) and no irrigation. Considerable variation exists in the physical structure, moisture condition, and topography of the soil in various parts of the orchard, so that it seems impossible to connect the disease with any of these factors.

Symptoms.—Most of the trees in this orchard have made very little growth, being not much larger after four or five years than when first planted (Fig. 2). Each spring vigorous new shoots start out, but in June the terminal buds wither and fall out and the terminal leaves turn a yellowish color. Often lateral shoots push out and then die back in the same manner. Eruptions of the bark and swollen and multiple buds occur, just as in the citrus disease (Fig. 3). The disease is not confined to prunes but shows itself on the same ranch to a greater or less extent on apples, pears, and Japanese plums. It also occurs on other ranches in the same vicinity.

During the spring of 1926 the following fertilizing materials were applied to rows of 16 trees each:

Row 3, ammonium sulphate 1 lb. a tree, sodium nitrate 1 lb., potassium sulphate 2 lbs., March 26.

Row 5, superphosphate 5 lbs. a tree, potassium sulphate 2 lbs., March 26.

Row 7, ammonium sulphate 1 lb. a tree, sodium nitrate 1 lb., superphosphate 5 lbs., March 26.

Row 9, ammonium sulphate 1 lb. a tree, sodium nitrate 1 lb., superphosphate 5 lbs., potassium sulphate 2 lbs., March 26.

[1] Of interest in this connection is the effect of copper sulphate on plant growth in peat lands. See Allison, R. V. et al. Stimulation of plant response on the raw peat soils of the Florida Everglades through the use of copper sulphate and other chemicals. Fla. Agr. Exp. Sta. Bul. 190: 35–80. 1927.

FIG. 2. Closer view of French prune trees with exanthema.

Row 11, superphosphate 5 lbs. a tree, Feb. 27.
Row 13, ammonium sulphate 2 lbs. a tree, Feb. 27.
Row 15, dried blood 4 lbs. a tree, Feb. 27.
Row 17, ammonium sulphate 2 lbs. a tree, superphosphate 5 lbs. a tree, Feb. 27.
Row 19, poultry manure 100 lbs. a tree, March 15.
Row 21, nitrate of soda 1 lb. a tree, March 26, and 1 lb. May 9.
Row 23, calcium nitrate 1 lb. a tree, March 26, and 1 lb., May 9.
Row 24, potassium sulphate 2 lbs. a tree, March 26.

In addition to this, four trees received an application of five pounds each of commercial copper sulphate on March 27. In each case the material was scattered about the tree and dug into the soil by hand.

During the season of 1926 no effect whatsoever could be seen in any of the trees except in the case of the four which received the copper sulphate. On these a vigorous, normal growth developed, with green foliage and ter-

FIG. 3. French prune twigs affected with exanthema; showing dead, swollen and multiple buds and exanthema of bark.

minal buds remaining throughout the season. The old bark lesions healed over, and the trunks and limbs became smooth and healthy looking. These four trees were outstanding in the orchard for their thrifty growth and appearance.

In the spring of 1927 four trees were treated with an application of 2½ lbs. each of c. p. copper sulphate, and ninety-six trees with 2½ lbs. each of commercial bluestone. Ten other trees received 25 lbs. each of double superphosphate, three trees 50 lbs. each of the same, and three trees 500 cc. each of commercial sulphuric acid, diluted with water to 5,000 cc. Again nothing showed the slightest effect except the copper sulphate. All the trees thus treated, except one or two which were nearly dead from the disease, responded markedly in growth, color, and healing of bark lesions. In August, 1927, measurements were made of the growth of leading shoots of many of the treated and untreated trees. On eight representative trees of those which had been treated with copper sulphate, 57 leading shoots averaged 37 inches growth in length in 1927. The maximum was 76 inches, and there were 14 shoots more than 50 inches long and 51 over 20 inches. On the nine best untreated trees adjacent to the others, the best 68 shoots averaged 13 inches growth in 1927. The maximum was 29 inches, and there were nine over 20 inches. At this time the treated trees were of a green, healthy color, with normal terminal buds. The untreated were chlorotic and sickly-looking and had lost practically all their terminal buds.

The four trees which were treated with copper sulphate in 1926 received no further applications but continued to grow normally and vigorously in 1927. Figure 4 shows two of these trees in August, 1927, together with some of the surrounding, untreated trees. The treated ones were rather worse than these before the application of copper sulphate.

EXANTHEMA IN APPLES

A very badly affected six-year-old apple tree stood in a garden on the same ranch. The whole top of this tree was a mass of brushy, yellow, die-back shoots. On June 23 a trench a foot deep was dug all around this tree, and two pounds of copper sulphate crystals sprinkled in the trench half way around the circumference of the tree. The trench was then filled with water, after separating the copper sulphate portion from the other half with earth. On August 17 a much improved condition was apparent all over the top of the tree on that side which had received the copper sulphate. On the untreated side the twigs were in even worse condition than before.

EXANTHEMA ON PEAR TREES

In another unirrigated region in California many Bartlett pear trees have typical symptoms of exanthema. Copper sulphate applied to the soil

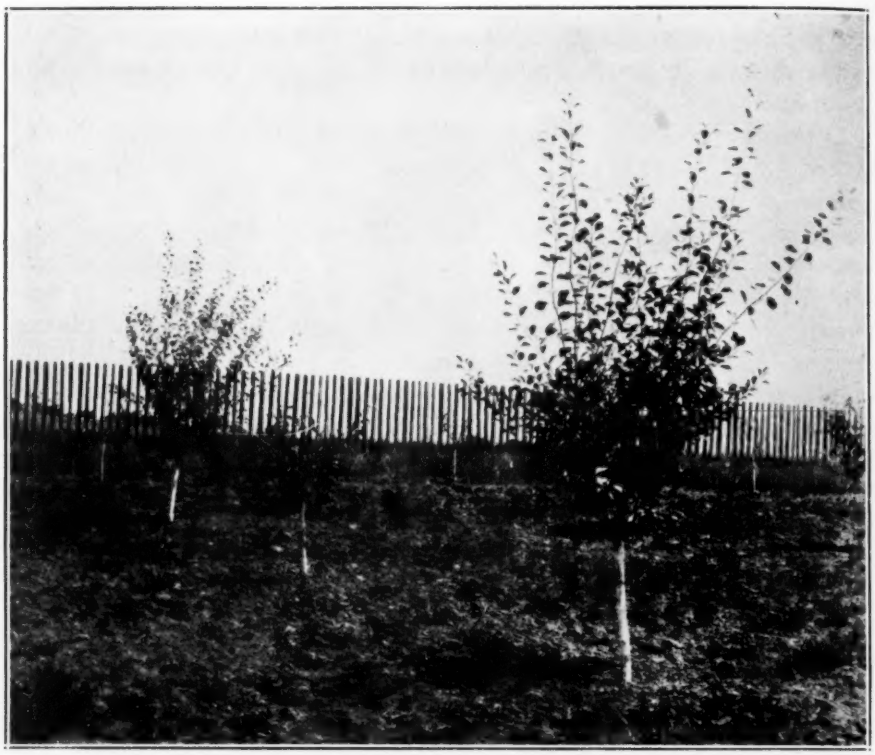

FIG. 4. Effect of copper sulphate on exanthema of French prune trees. Five pounds a tree was applied to alternate trees the previous year.

about some of these trees in the spring of 1927 brought about as decisive an improvement as that in the case of the prune and apple trees.

EXANTHEMA IN OLIVES

In still another district, typical exanthema symptoms occur quite abundantly on young olive trees. The terminal buds and shoots wither and die, laterals push out and meet the same fate, and eruptions develop in the bark. In this case the trouble is mostly confined to spots in the orchard which become very wet during rainy weather and irrigation, and very dry and hard between times. Trial of copper sulphate was suggested in this case, and growers have reported beneficial results.

Investigations on the soils of these different localities, which appear to vary widely from one another in many respects, both physical and chemical, are being conducted by the division of plant nutrition.

DIVISION OF PLANT PATHOLOGY,
COLLEGE OF AGRICULTURE,
UNIVERSITY OF CALIFORNIA.

CLASSIFICATION OF COPPER FUNGICIDES[1]

E. B. Holland and G. M. Gilligan

In considering the numerous copper fungicides that have been recommended by different investigators, or introduced by various manufacturers, sufficient diversity will be observed to warrant a classification, first, based on the mode of application, into (A) sprays and (B) dusts; and secondly, based on the solubility of the product at the time of application, into (I) soluble and (II) insoluble. Such a classification is necessarily more or less arbitrary and lacks scientific precision, but has proved extremely serviceable.

With sprays, the vehicle is water and the soluble forms of copper are true solutions of simple salts or cuprammonium compounds and the insoluble forms are colloids (suspensoids or emulsoids) or suspensions (fine or coarse). The soluble forms of copper are immediately active, toxic to foliage, especially under slow drying conditions, easily distributed, and the residue on evaporation inconspicuous and poorly adhesive. The adhesiveness to the foliage depends mostly on the chemical composition. A deposit of copper sulphate remains soluble and is readily removed by rain. The cuprammonium compounds are probably rendered insoluble more rapidly than the acetates, but the latter, particularly the basic, are more persistent on the leaves. Occasionally substances are added to Bordeaux or Burgundy mixtures with a view of producing soluble derivatives of copper and thereby increasing the activity of the mixture. Of these, sugars and some hydroxy-organic acids are the most promising, although the practice is not recommended.

The insoluble forms of copper as a class are slowly active, comparatively non-toxic to the plant, more difficult to apply, and the residue on evaporation readily visible and adhesive. The adherence depends largely on the physical characteristics, and the colloids and gelatinous products are considered the most efficient. The insoluble forms of copper employed are generally basic sulphates or basic carbonates, either home-made or commercial. Cupric hydroxide and so-called colloidal copper have recently been offered for trial by the producers. The efficiency of all insoluble deposits of copper, whether natural or air-converted, depends on a small amount being rendered soluble by atmospheric agents, osmosis or other factors

[1] From the Department of Chemistry, Massachusetts Agricultural Experiment Station. Printed with the permission of the Director of the Station.

The writers wish to acknowledge the assistance of Dr. Paul Serex, of the Department of Chemistry of the Massachusetts Agricultural College.

acting singly or jointly. Substances are often added with a view of increasing the degree of suspension, assuring more uniform distribution (spreading) and increasing adhesiveness. As the addition of such materials generally tends to lower the availability of the copper by reducing the concentration of the residue, or by forming less ionizable derivatives, only a small amount of such substances (0.10 per cent of the spray or less) should be employed.

With dusts the vehicle may be a relatively inert carrier such as talc, steatite, kaolin, clay, Fuller's earth, infusorial earth, pumice, gypsum, coal dust, sulphur, aluminum hydroxide, or a more reacting (protective) carrier such as calcium carbonate or calcium hydroxide. The soluble forms of copper may be saturated solutions or pulverized, partially dehydrated or anhydrous salts, generally the sulphate, incorporated with an inert vehicle. With a reacting vehicle in the presence of moisture, a portion of the copper at least would be rendered insoluble. This sub-group might be omitted on the grounds that most of the copper present is either soluble or insoluble on application, but, as some of the mixtures occupy an intermediate position, it is retained. The insoluble forms are copper stearate and those mentioned under sprays.

The following tentative classification is presented:

A. Sprays.
  I. Soluble—True solutions.
    (a) Simple salts.
      (1) Copper sulphate ..... $CuSO_4 \cdot 5H_2O$.
      (2) Normal copper acetate (verdet neutre) $(CH_3COO)_2Cu \cdot H_2O$.
      (3) Basic copper acetate[2] (verdet gris) $(CH_3COO)_2Cu \cdot CuO \cdot 6H_2O$.
    (b) Cuprammoniums.
      1. Ammonia (hydroxide) soluble.
        (1) Copper sulphate (eau celeste).
        (2) Cuprammonium sulphate ..... $Cu(NH_3)_4SO_4 \cdot H_2O$.
        (3) Burgundy mixture (modified eau celeste).
        (4) Malachite (cupram).
      2. Ammonium carbonate or bicarbonate soluble.
        (1) Copper sulphate (Johnson's mixture).
        (2) Cuprammonium sulphate.
        (3) Burgundy mixture.
        (4) Malachite.
    (c) Organic copper derivatives in alkaline mixtures (Bordeaux. Burgundy) produced by:
      (1) Sugars, dextrin, glycerol, etc.

[2] The basic portion is probably a suspensoid.

(2) Hydroxy-organic acids.
(3) Biuret compounds (probably colloidal).

II. Insoluble.
(a) Colloids.
1. Suspensoids.
(1) Colloidal copper.
2. Emulsoids.
(1) Pickering spray emulsion.
(2) Bordo emulsion.
(3) Cuprammonium sulphate emulsion (Kupfer preparat Gmund).
(b) Suspensions.
1. Fine (gelatinous, often termed colloidal).
(1) Bordeaux mixture.
Highly basic, basic and alkaline.
Neutral.
Acid (flocculent precipitate).
(2) Pickering sprays.
Lime water precipitated.
Barium water precipitated.
(3) Burgundy mixture (soda Bordeaux).
(4) Sodium bicarbonate Bordeaux.[3]
(5) Mixed carbonates Bordeaux.[3]
(6) Bordeaux paste.
(7) Colloidal copper (proprietary).
2. Coarse.
(1) Dry Bordeaux (Strawsonite).
(2) Basic copper sulphate ..... $CuSO_4 \cdot 3CuO \cdot 4H_2O$.
(3) Copper hydroxide.

B. Dusts.
I. Soluble.
(a) With inert vehicles.
(1) Sulphosteatite (Fostite).
(2) Various French and German mixtures (copper sulphate, etc.).
(3) Sulphated sulphur (Blight powder).
(b) With more or less reacting vehicles.
(1) Sulfatine.
(2) Several foreign mixtures (copper sulphate, lime, etc.).
(3) Monohydrated or anhydrous copper sulphate and hydrated lime.

II. Insoluble.
(1) Dry Bordeaux.
David powder, Podechard powder, Cuprosteatite, etc.
(2) Basic copper sulphate ..... $CuSO_4 \cdot 3CuO \cdot 4H_2O$.
(3) Malachite (disinfectant) ..... $CuCO_3 \cdot Cu(OH)_2$.
(4) Copper stearate ..... $(C_{17}H_{35}COO)_2Cu$.

[3] Contains more or less soluble copper.

It is to be regretted that all of the products cannot be listed as chemical entities, but this is impossible from present knowledge. Furthermore, the lack of control measures in the preparation or manufacture of the different products makes it impossible to assure always a single active compound, and often there is produced a mixture together with an excess of precipitant and of more or less inactive by-products. The principal objective is to obtain an active product of good physical characteristics suitable for application as a spray or as a dust. Any classification based on one or two characteristics must necessarily show inconsistencies in other respects unless the subdivisions are increased to a point unwarranted in this connection. Innumerable other compounds, preparations, and modifications of sprays and dusts have been mentioned in literature, but, seemingly at least, are not of sufficient importance to be listed.

DEPARTMENT OF CHEMISTRY,
MASSACHUSETTS AGRICULTURAL EXPERIMENT STATION,
AMHERST, MASS.

PHYTOPATHOLOGICAL NOTES

The transmission of potato black-leg by the seedcorn maggot in Maine. Proof by Leach that the seedcorn maggot (*Hylemyia cilicrura* Rond.) disseminates potato black-leg in Minnesota[1] was followed by observations on this phenomenon in Aroostook County, Maine.

Flies of this species were collected in abundance in 1925, 1926, and 1927. In 1927 a very severe black-leg epiphytotic occurred. Many unexplained cases of high black-leg percentage were observed in fields planted with seed potatoes thought to have been disease-free. On June 20 a collection of the adult flies was made in a fallow field. Some of them were kindly identified by Dr. O. A. Johannsen of Cornell University. The rest were placed in 8 covered glass jars containing sterilized soil and healthy seed pieces from fungicide-treated tubers. Each jar contained 5 seed pieces, partly covered by the soil, and from 3 to 5 of the introduced flies. The seed tubers were all represented by seed pieces in 2 similar jars without flies, these serving as controls. About 4 weeks later a rapid seed-piece rot developed in 3 of the jars containing insects. In these 3 jars numerous maggots were burrowing into the rotted seed pieces. The jars were kept in the light and the soil was kept watered. Of the 15 seed pieces contained in the 3 jars infected with maggots, 5 were destroyed before producing plants, while the other 10 produced 10 black-leg plants in the jars. The flies failed to produce maggots in 5 jars, the cause not being understood. The seed pieces in these 5 jars and in the 2 control jars remained healthy and produced healthy plants.

Some of the maggots were placed in proximity to other freshly cut potato seed pieces. The maggots soon attacked this potato material through the cut surfaces and burrowed deeply, initiating a rapid soft rot like that caused in the preceding experiment.

Field observations revealed that the same type of maggots were common in the soil beneath black-leg plants. Collections of these maggots were also capable of causing a rapid rot in freshly cut potato tubers.

From these results it is concluded that the seedcorn maggot is a factor to be considered in the control of potato black-leg in Maine.—REINER BONDE, Agricultural Experiment Station, Orono, Maine.

[1] Leach, J. G. The seed-corn maggot and potato blackleg. Science **61**: 120. 1925. ———. The relation of the seed-corn maggot (*Phorbia fusciceps* Zett.) to the spread and development of potato blackleg in Minnesota. Phytopath. **16**: 149–175. 1926.

Bacterium stizolobii (Wolf) comb. nov. syn. Aplanobacter stizolobii In the original publication on this plant pathogen (Phytopath. 10: 73–80. 1920) it is described as non-motile and Morrey's modification of Loeffler's method failed to demonstrate any flagella. In a later study by the writer a single, short, polar flagellum was stained both by the Casares-Gil and by David Ellis methods. The organism is apparently non-motile in most cultures, also when examined directly from leaf lesions. Slight but definite motility was observed in some young, beef-agar cultures, and from these the flagella were demonstrated.—LUCIA MCCULLOCH, Department of Agriculture, Washington, D. C.

Bacterium maculicola (McC.) nom. emend. syn. Bacterium maculicolum. Original publication: A spot disease of cauliflower, U. S. Dept. Agr., Bur. Plant Indus., Bul. 225. 1911. The ending *a* is the preferred spelling.—LUCIA MCCULLOCH, Department of Agriculture, Washington, D. C.

A question of correct usage. It is quite the usual thing for beginners in biologic science to find Latin plurals a stumbling block, but it seems that many experienced workers are tripping over the same stone in their published scientific contributions. I refer to the frequent misuse of the Latin plural form, especially the plural ending in –a, in forming compound words where ordinary English usage calls for the singular. To illustrate: When we refer to fungous hyphae which bear spores we properly speak of "spore-bearing" hyphae. When we wish to refer to hyphae which bear conidia the proper expression is "conidium-bearing," but frequently the combination "conidia-bearing" is used instead. In the same way "bacteria-like" is used where "bacterium-like" ought to be employed. The absurdity of these and other similar abnormal combinations is perfectly apparent if we listen to the odd sound of their approximate equivalents, e.g.—spores-bearing, germs-like.—H. P. BARSS, Oregon State Agricultural College, Corvallis, Oregon.

Notice of field meeting of the Southern Section of the American Phytopathological Society. At the Nashville meeting of the American Phytopathological Society the members of the Southern Section voted unanimously to hold a field meeting at a time and place to be decided upon later. Accordingly arrangements have been made for a three-day field meeting to be held in Georgia, July 11–13, 1928, and a cordial invitation to attend is extended to all who are interested in diseases of southern crops. The Georgia plant pathologists have planned an excellent program with a num-

ber of unique and valuable features, and it is hoped that a large number will plan to attend.

Briefly, the itinerary outlined is as follows:

July 11. Assemble at Experiment, Georgia, at 10 A. M. See the research work of the Experiment Station, including results of cotton seed treatments, work on pepper diseases, tomato wilt, peach rosette, peanut diseases, and other plant diseases. Attend the dedication of the new library and laboratory building of the Experiment Station and barbecue at noon. 8 P. M. Discussion of the day's observations and plans for the following day.

July 12. Drive 64 miles to Fort Valley. See the work of the United States Peach Insect and Disease Laboratory on peach insects, brown rot, bacterial spot, and "Phony" peach. Visit some of the commercial orchards and packing houses. Drive 80 miles to Albany, and spend the night there. 8 P. M., assemble for discussion, etc.

July 13. See airplane dusting of pecan grove. See some of the principal pecan diseases and control methods as practiced in commercial groves about Albany. Drive 45 miles to the Coastal Plain Experiment Station, Tifton, to see the work on cantaloupe and tobacco diseases. Drive 50 miles to Thomasville to see the work on pecan and other crop plant diseases being done at the United States Pecan Disease Laboratory and at the Field Laboratory of the State Board of Entomology.

The trip will end officially at Thomasville, Georgia, but arrangements have been made for those interested in tobacco diseases to visit the Florida Tobacco Station, Quincy, Florida.

Automobile transportation will be furnished locally for visitors who do not find it convenient to bring their own cars. Those planning to attend are asked to communicate as soon as possible with Dr. B. B. Higgins, Agricultural Experiment Station, Experiment, Georgia, indicating whether or not they desire transportation furnished for the field trip.—V. H. YOUNG, Secretary, Southern Section, American Phytopathological Society.

REPORT OF THE NINETEENTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

ADDITIONS AND CORRECTIONS TO REPORT OF EIGHTEENTH ANNUAL MEETING

1. The following paragraph was inadvertently omitted from the Secretary's report of the Philadelphia meeting (Phytopath. 17: 343–353. 1927.)

> The matter of a permanent endowment for PHYTOPATHOLOGY was proposed by E. C. Stakman. It was pointed out that the journal has practically reached the limitations made possible by its present income and that more money is needed for expansion. It was voted to have a committee on permanent endowment and the newly elected President later appointed E. C. Stakman, C. R. Orton and I. E. Melhus. The fund was started by a twenty-dollar check from H. H. Whetzel.

2. In the report of the Business Manager of PHYTOPATHOLOGY the statement of liabilities (page 347) should read "Outstanding checks—$914.98" instead of "none" as printed.

THE NASHVILLE MEETING

The joint annual meeting of the American Phytopathological Society and its Southern Division was held at Nashville, Tennessee, December 28–30, 1927, in conjunction with the American Association for the Advancement of Science and affiliated societies. The hotel headquarters were at the Sam Davis Hotel and the meeting rooms were in the Demonstration School of the George Peabody College for Teachers. About 150 members were in attendance.

The program was made up of 90 papers, 18 in general sessions, 4 in joint session with Section G, 12 in joint session with the Mycological Section of the Botanical Society of America, and the remainder in sessions for papers on tobacco diseases (8), diseases of southern crops (7), cereal and forage crop diseases (11), potato and vegetable diseases (11), fruit diseases (9), diseases of ornamental plants (7), and diseases of sweet potatoes (3). Of the 90 papers, 82 were contributed by this Society, 4 by the Mycological Section, and 4 by Section G.

At a conference on market pathology and its relation to extension activities, talks were given on various phases of the work by F. C. Meier, Chairman; William Turner, Horticultural Agent of the Central of Georgia Railroad; F. G. Robb, of the U. S. Bureau of Agricultural Economics; J. I. Lauritzen and R. J. Haskell, of the U. S. Bureau of Plant Industry; V. H. Young, of the Arkansas Agricultural Experiment Station; J. J. Taubenhaus, of the Texas A. & M. College, and others.

At the Plant Disease Survey supper and round table, the recent surveys in Missouri, Iowa and Utah were discussed.

The Phytopathologists' dinner was held Thursday evening, December 29, at the Nashville Chamber of Commerce. President Barrus, being located in Porto Rico for a year, found it impossible to be in attendance. Therefore Vice-President Barss, of Oregon, officiated both at the dinner and at the other sessions of the Society. Dr. L. R. Hesler was in charge of the arrangements for the after-dinner program which, guided by our efficient and effective Vice-President, proved very entertaining. A quartet of real southern negro singers, an interesting letter from President Barrus read by Dr. Mel T.

Cook, an impersonation of the ghost of Charles Darwin, by F. D. Fromme, a talk by Watson Davis of Science Service, and the reading of, and awarding of prizes for phytopathological limericks by Drs. E. C. Stakman and L. R. Jones made up the program.

OFFICERS AND REPRESENTATIVES

The following officers were chosen:

President, H. P. Barss, Oregon Agricultural College, Corvallis, Ore.

Vice-President, F. D. Heald, Agricultural Experiment Station, Pullman, Wash.

Councilor (two years), F. D. Fromme, Virginia Polytechnic Institute, Blacksburg, Va.

Editor-in-Chief of Phytopathology (three years), E. C. Stakman, University of Minnesota, University Farm, St. Paul, Minn.

Editors (three years), H. M. Quanjer, Editor for Europe, Institut voor Phytopathologie, Wageningen, Holland; L. R. Hesler, University of Tennessee, Knoxville, Tenn.; M. N. Levine, University of Minnesota, St. Paul, Minn.

Associate Editors (three years), W. B. Tisdale, Tobacco Experiment Station, Quincy, Fla.; S. M. Zeller, Oregon Agricultural College, Corvallis, Ore.; Margaret Newton, Manitoba Agricultural College, Winnipeg, Canada; C. W. Bennett, Michigan Agricultural College, East Lansing, Mich.; Donald Folsom, Maine Agricultural Experiment Station, Orono.

Business Manager (one year), R. J. Haskell, Bureau of Plant Industry, Washington, D. C.

Advertising Manager (one year), J. F. Adams, Agricultural Experiment Station, Newark, Del.

Representatives on the Council of the American Association for the Advancement of Science (one year), Donald Reddick, New York State College of Agriculture, Ithaca, N. Y., and C. W. Edgerton, Louisiana Agricultural Experiment Station, Baton Rouge, La.

Members of the Advisory Board (three years), J. A. Stevenson, Bureau of Plant Industry, Washington, D. C., to succeed F. C. Meier as Commissioner for the U. S. Department of Agriculture; F. L. Drayton, Dominion Department of Agriculture, Ottawa, Canada, to succeed J. E. Howitt as Commissioner for Canada; and I. E. Melhus, Iowa State College of Agriculture, Ames, Iowa, to succeed M. F. Barrus as Commissioner at large.

Members of the Board of Governors of the Crop Protection Institute (three years), J. F. Adams, Agricultural Experiment Station, Newark, Del., to succeed N. J. Giddings. The other two members of the board are I. E. Melhus and H. W. Anderson.

Representative (Elector) on the Division of Biology and Agriculture of the National Research Council (three years), F. D. Fromme, Virginia Polytechnic Institute, Blacksburg, Va.

Member of the Permanent Committee on Necrology, M. B. Waite, Bureau of Plant Industry, Washington, D. C., to succeed Haven Metcalf, resigned. The other members of this committee are G. P. Clinton and L. R. Jones.

The following temporary committees were appointed by the President to serve throughout the meetings: *Resolutions Committee*, J. C. Walker, A. G. Johnson and C. R. Orton; *Auditing Committee*, W. H. Tisdale and B. F. Dana; *Committee on Publicity*, F. J. Schneiderhan, J. F. Adams, and G. H. Coons; *Committee on Elections*, L. M. Massey, R. S. Kirby and M. W. Gardner.

The Southern Division of the Society chose the following officers for 1928 at its business session, December 29. President, B. B. Higgins; Secretary, V. H. Young; Councilor, W. B. Tisdale.

REPORT OF THE SECRETARY TREASURER, 1927

At the close of the Philadelphia meeting the membership totaled 716. During the year a loss of 31 members was sustained—22 by suspension for non-payment of dues, 7

STATEMENT OF ACCOUNTS FOR 1927, AS OF DECEMBER 19, 1927

Receipts:

Balance from 1926		$2,129.06	
Annual dues: 1924	$ 4.00		
1925	14.00		
1926	32.00		
1927	1,075.70		
1928	1,719.21		
1929	13.50		
		2,858.41	
Excess dues		1.49	
Interest on checking account		19.24	
Interest on time deposit		30.00	
Cash returned by Secretary-Treasurer, expenses of meeting		9.17	
Dues in Physiological Section received with annual dues		1.50	
Sales received with annual dues		5.62	
			$5,054.49

Expenditures:

Member subscriptions transferred to PHYTOPATHOLOGY	$1,962.00	
Secretarial work	295.75	
Postage stamps	39.00	
Telegrams	2.47	
Printing (preliminary announcements, dinner tickets, programs, application blanks, ballots, nomination ballots, envelopes)	169.50	
Expense of lantern slides of seals of Society	5.55	
Expenses of Secretary-Treasurer at annual meeting	75.00	
Work on Erwin F. Smith testimonial	36.50	
Miscellaneous expenses (account cards)	.94	
Dues in Physiological Section paid	1.50	
Checks returned by bank	12.00	
Amount transferred to Sinking Fund for investment	498.00	
		$3,098.21
Balance on checking account		$1,956.28
Outstanding check		43.50
		$1,999.78
Amount of above receipts credited to 1928–1929	$1,732.71	
Amount due Sinking Fund	24.00	
Sales due PHYTOPATHOLOGY	5.12	
		$1,761.83
Actual balance for 1927		$ 237.95

by resignation, and 2 by death, making the total membership 685 at the close of 1927. Of these, 74 were life members paid in full, 39 were life-sustaining members paying currently, and 572 were annual regular members. At Nashville 65 new members were elected, thus bringing the total up to 750, a net gain of 34.

In the United States, New York and Minnesota tied for first place in the securing of new members with 7 each. Wisconsin and Iowa tied for second place with 5 each. These were followed by Florida with 3, California and Delaware with 2, and 18 other states with one each. Of the foreign countries Japan led with 6 new members, then Canada with 4, England and Australia with 2 each, and Czechoslovakia and India with one each.

The increase of members in foreign countries during the recent years is notable. In 1924, 8 foreign members were elected, in 1925—9, in 1926—19 and in 1927—17.

REPORT OF THE BUSINESS MANAGER OF PHYTOPATHOLOGY FOR 1927

Since June, 1924, PHYTOPATHOLOGY has been printed by the Science Press Printing Company, of Lancaster, Pa., and it is generally agreed that a very creditable piece of work has been done. It is with deep regret that we report the death on February 3, 1927, of the Manager of the Company, Mr. A. E. Urban, who took a great personal interest in the welfare of the journal. A printer by trade and a botanist at heart, he was especially fitted to handle and to appreciate our work. Since his death the business has been efficiently carried on by Mr. Jaques Cattell, Secretary of the Company, and his associates.

The close of the year finds PHYTOPATHOLOGY not quite so well off financially as in other recent years, the bank balance being $585.08 as compared with $1,103.84 last year. This seems to be due principally to decreased receipts from sales of back numbers and advertising. In addition to the balance of $585.08 and exclusive of the sinking fund the journal has $1,000 invested in securities.

The number of subscribers in good standing at the close of the year is 420, of which 252 are foreign and 168 domestic. The present edition of PHYTOPATHOLOGY is 1,525 copies, of which 1,136 are being mailed out.

The sinking fund now amounts to $5,274.00.

STATEMENT OF ACCOUNTS FOR 1927, AS OF DECEMBER 19, 1927

Receipts:

Balance from 1926		$1,103.84
Subscriptions ($686.40 for 1928, $2.50 for 1929)		2,483.97
Sales, PHYTOPATHOLOGY		370.29
Phytopathological Classics		112.18
Advertising: 1926	$146.08	
1927	719.53	865.61
Interest on Sinking Fund and investment		392.32
Donation to PHYTOPATHOLOGY Endowment Fund		20.00
Amount due Sinking Fund from Society		498.00
Member subscriptions for 1927		1,962.00
Sinking Fund investment paid up (reinvested)		500.00
		$8,308.21

Expenditures:

Manufacturing PHYTOPATHOLOGY:			
Vol. XVI, No. 12	$ 635.65		
Vol. XVI, Index	138.66		
		$ 774.31	
Vol. XVII, No. 1	382.71		
Vol. XVII, No. 2	450.63		
Vol. XVII, No. 3	430.14		
Vol. XVII, No. 4	366.09		
Vol. XVII, No. 5	589.42		
Vol. XVII, No. 6	391.44		
Vol. XVII, No. 7	553.71		
Vol. XVII, No. 8	360.95		
Vol. XVII, No. 9	570.63		
Vol. XVII, No. 10	572.05		
Engravings for Vol. XVII	796.09		
		$5,463.86	
			$6,238.17
Manufacturing Phytopathological Abstracts			26.80
Manufacturing Phytopathological Classics			232.90
Expenses of Editor-in-Chief (editing manuscripts, etc.)			65.62
Expenses of Advertising Manager (postage, stamped envelopes, stenography)			43.62
Postage, Business Manager			15.00
Miscellaneous expenses (account books, expressage)			2.10
Secretarial work			91.50
Sinking Fund invested and reinvested with accrued interest			$1,006.42
Purchase copy of PHYTOPATHOLOGY			1.00
			$7,723.13
Balance			$ 585.08
Outstanding checks			49.64
			$ 634.72

Assets:

Cash in bank (less outstanding checks)	$ 585.08	
Sinking Fund (invested)	5,250.00	
Amount due Sinking Fund (now in Society's account)	24.00	
First mortgage (surplus)	1,000.00	
Amount due from subscriptions	826.50	
Amount due from advertising	202.40	
Amount due from sales made	20.22	
Due from Society for sales included with dues	5.12	
Checks on hand to be deposited	140.15	
		$8,053.47

Liabilities:

None.

REPORT OF THE EDITOR-IN-CHIEF OF PHYTOPATHOLOGY

Volume 17 of PHYTOPATHOLOGY contains 836 pages, comprising 69 articles, 15 notes, 3 reports, and 3 book reviews; 31 plates and 169 text figures. Ninety-eight manuscripts were submitted, of which 22 were returned for revision and 4 rejected. The papers published in Volume 17 have been, on the average, shorter than those published in 1926. Fewer papers were rejected, although a considerable number had to be returned to the authors for major revision or for approval of changes made by the editorial staff. Volume 17 contains a larger number of illustrations than the previous volume, which will probably mean a greater expense to the Society.

In our report last year it was stated that five months, the average length of time required to get a paper into print, probably could not be shortened very much. During 1927, however, we have been able to publish manuscripts, on the average, in four months from the date of receipt. A few have required six months; a few have been published in two months; the number which required five months is balanced by those published in three months; and the larger number by far have appeared in four months. We have accomplished this by putting into each number most of the galleys on hand. This procedure has several disadvantages: it puts a burden on the printer by requiring him to print material as soon as it is received; we lead a somewhat hand-to-mouth existence by enriching one number and impoverishing the next; and on both of these accounts numbers are likely to be behind schedule and unequal in size. Only by doing this, however—by using all of the galleys available when a number is being made up—has it been possible to shorten the time required for publication.

Acknowledgement is due each of the associate editors for indexing one number of Volume 17. Some of the associate editors, in addition, have rendered very valuable service in approving or rejecting manuscripts submitted to them for transmittal to the editor-in-chief. For this assistance the editor-in-chief is very grateful.

According to the editorial committee for Phytopathological Abstracts, the papers submitted for the program of the annual meeting were a great deal too long, in many cases, to come within the 200-word limit. The editorial committee had insufficient time to return the abstracts to authors; neither could they take the time, nor the risk, to condense papers enough to make them come within the word limit. Some of the abstracts were over 300 words in length. There seems to be little excuse for this. The committee therefore recommended that the procedure for next year be changed: the abstracts should be requested earlier, say November 1; the editorial committee would return those which did not come within the word limit, and those which do not offer worth-while information. By moving the date of receipt forward, there would be time to edit the abstracts carefully, and time to satisfy the committee and the authors in regard to points of conflict.

Detailed editing of all manuscripts has been done by Helen Hart and Laura M. Hamilton. They wish to call the attention of authors again to the advantage of well-prepared papers in respect to date of publication. If changes seem desirable, and if the paper must be returned to the author for approval of changes, it means delayed appearance of the manuscript.

They find the most difficulty with tables. Headings are incomplete, column headings often are obscure, and the form does not correspond with that used in PHYTOPATHOLOGY. A table should be as nearly self-explanatory as possible, for it is not always possible to place it immediately after the reference in the text. Examination of recent numbers of PHYTOPATHOLOGY would be helpful to many authors in determining what is required in tables, and would mean that their papers could be published earlier than they are.

Graphs also are frequently submitted which are unsuitable for satisfactory reproduction. When graphs on finely squared paper are reduced to page width the background often appears as indistinct grayish blocks. Plain paper or paper ruled in large squares gives more satisfactory results. All too often, also, letters and figures are so small that they can be distinguished only at the risk of permanent eye-strain. And they should never be pasted on.

Authors of scientific papers should be as accurate in expressing their ideas and the results of their experiments as they are in making their tests and in drawing conclusions. Clear writing—it need not be polished—is as much a part of "scientific accuracy" as the details of the experiments which the reader does not see.

A few avoidable errors have appeared in Volume 17: for these the editorial staff makes apology to the authors concerned.

REPORT OF THE ADVERTISING MANAGER

The advertising returns have been disappointing for the fiscal year of 1927. Instead of the increased financial returns we have experienced for the past two years, which were expected to be maintained, the record shows a decrease of business for the first time. A total of 113 advertisements appeared in the 12 issues for 1927 or a decrease of 27.5 per cent compared with last year. This total of advertisements was distributed as follows: 41 full pages, 39 half pages, 32 one-fourth pages, and 1 one-eighth page, making a total of 68⅝ pages of advertising or a decrease of 23½ pages. Three clients who have been placing a total of 30 pages failed to renew contracts for business reasons. If their business had been maintained it would have been possible to report another year of increased business.

It is desired further to impress the members that their cooperation is essential in order to increase our list of clients. When being solicited or when in contact with representatives of prospective commercial companies, make it a point to mention the merits of our publication as a practical medium in which to advertise. The personal appeal and contact is the most productive means of securing business. Our membership is sufficiently large and distributed so that with a little cooperation our business could easily be doubled.

REPORT OF THE ADVISORY BOARD

The personnel of the Board for 1927 is as follows: F. D. Fromme, Chairman, representative at large; F. C. Meier, Secretary, representing the United States Department of Agriculture; M. F. Barrus, representative at large; J. G. Dickson, representing the Midwest; B. B. Higgins, representing the South; J. E. Howitt, representing Canada; E. L. Nixon, representing the Northeast; and S. M. Zeller, representing the West. The following appointments were made by the Council for 1928: I. E. Melhus to succeed M. F. Barrus; J. A. Stevenson to succeed F. C. Meier; and F. L. Drayton to succeed J. E. Howitt.

F. D. Fromme was elected Chairman of the Board for 1928, and J. G. Dickson was elected Secretary.

Summer Meetings. The summer meeting for 1927 was held August 16–19 and included a tour of northern Ohio for the study of diseases of fruits, vegetables, and other crops. The local committee on arrangements consisted of H. C. Young, Chairman, W. G. Stover, Curtis May, Paul E. Tilford, and R. C. Thomas. The meeting was well attended and was unusually successful, due to the excellent program and arrangements provided by the local committee. The itinerary included the study of experimental

plots and commercial production of a considerable variety of crops such as potato, cucumber, tomato, corn, oats, onion, celery, raspberry, peach, and grape. Occurrence of disease on a number of additional crops was also noted. A more complete report of the meeting appears in Phytopath. 17: 836. 1927.

At the annual meeting of the Board it was decided to hold a summer meeting in 1928 on diseases of ornamental plants in the vicinity of New York City. A local committee on program and arrangements consisting of W. H. Martin, Chairman, L. O. Kunkel, C. R. Orton, W. H. Rankin, and L. M. Massey has been appointed, and plans are now being formulated.

National Research Council. The Division of Biology and Agriculture of the National Research Council has submitted a statement of the activities that are of particular interest to this Society, in order that the members may be informed of the types of service that may be rendered. A brief summary of the statement follows:

The Division through its various committees has aided the establishment of the Crop Protection Institute, the Tropical Plant Research Foundation, and the Barro Colorado Island Biological Station. It has given financial and other aid to *Botanical Abstracts*, *Biological Abstracts* and the Arthur Rust project.

It has had in operation since 1923 the National Research Fellowships in Biological Science. The fellowships of especial interest to this Society are the hard-seed fellowship which is in active operation under the direction of Dr. B. M. Duggar, and the sulfur fellowships which have largely been completed.

The Committee on Tropical Research has had under advisement the establishment of a graduate school of tropical agriculture and is now recommending Porto Rico as the most suitable location for the proposed school.

The Committee on Research Publications is determining the needs among scientific biological journals for the publication of original research.

"On behalf of the Division of Biology and Agriculture, its present officers wish to call the attention of the members of the American Phytopathological Society to the fact that the National Research Council welcomes the representation on it of the Society and that the Division is the unit through which the Society secures this representation and to whom it may bring any matters in which the Council can be of assistance. The extent to which this Society, along with the others included in this one unit, utilizes the Division organization as a means to accomplish some needs, is the extent to which the Division itself will justify its value to the societies."

Crop Protection Institute. The activities of this organization, which cooperates with the Advisory Board, are summarized from a report of W. C. O'Kane, Chairman of the Board of Governors, as follows:

At the close of 1927 the Institute had a staff of fifteen research workers, all but two of whom were full-time men. Fifteen research projects were in operation. These are listed briefly as follows:

Control of crown gall. At Ames, Iowa; Madison, Wisconsin; and Yonkers, New York.

Plant diseases carried by seeds. At Yonkers, New York.

Thallium and mercury compounds. At Yonkers, New York.

Insecticidal possibilities of the white oil emulsion, Volck. At Manhattan, Kansas.

Fungicidal action of a new colloidal sulfur. At Urbana, Illinois, and Madison, Wisconsin.

New colloidal copper compounds. At Yonkers, New York, with supplementary work in various states.

New compounds derived from furfural and furfuramid. At Ames, Iowa, and Fort Collins, Colorado.

Oil compounds as cattle sprays. At Lafayette, Indiana.

Insecticidal development of the household spray, "Kip." At Lafayette, Indiana.

Development of new oil emulsions. At Urbana, Illinois.

Development of the household spray, "Flit." At New Brunswick, New Jersey.

New horticultural sprays. At New Haven, Connecticut.

The physical character of atomized sprays. At New Brunswick, New Jersey.

Oxidized oils as insecticides. At Lake Alfred, Florida.

Organic insecticides. At New Brunswick, New Jersey.

Duties of the Advisory Board. The Advisory Board was formally established by the Society in 1918. A restatement of the duties as formulated at that time should be worth while. They are as follows: (1) To represent the Society before the National Research Council, (2) To prepare and distribute to the members an annual list of the active phytopathological projects in progress in the country, (3) To arrange for conferences of groups of workers on related subjects, (4) To confer with workers in related fields in order to promote joint efforts on our common problems, (5) To promote international relations in phytopathology, (6) To take up such other problems as the Board may find necessary, subject to the approval of the Council.

The recent activities of the Board, as will be noted, have dealt largely with the first, third, and fourth of these duties. No list of active projects has been prepared in recent years, and apparently there has been little need or demand for such a list. The Board has been, and is, successful in its chief function which is the promotion of cooperation. It will be glad to render any further service within its power that the Society may direct.

REPORTS OF OTHER COMMITTEES AND REPRESENTATIVES

Representative on American Type Culture Collection. In the absence of the Society's representative, C. L. Shear, the following report was submitted by Dr. Margaret B. Church. A supplementary list of available fungous cultures was distributed at the meeting.

"The first catalogue of the American Type Culture Collection, issued in 1927, lists about 230 fungous cultures in the group cared for by Drs. Charles Thom and Margaret B. Church of the United States Department of Agriculture. This group excludes human and animal pathogens and Actinomyces. Sixty-five per cent of the fungi included were contributed by Thom and Church, and the remaining 35 per cent by others. Since this catalogue went to press there have been added 130 new cultures now listed on a supplementary sheet. Of these new fungous cultures, 57 per cent were secured through the efforts of Drs. Thom and Church because of established personal relations, 24 per cent were secured through the efforts of their colleagues, 9 per cent were from their personal collection, while 7 per cent were from Dr. Shear, and 3 per cent from odd sources.

"It was the understanding of Drs. Thom and Church in December, 1924, that they should supervise constantly and critically the care and distribution of these fungous cultures after once formulating a standard of work. Accordingly, their efforts to secure cultures have been incidental to their official relations in the field of soil, food, and industrial mycology.

"An assistant is financed by the General Education Board and the Society of American Bacteriologists. The hours of work are arranged individually with each new assistant. The present incumbent, Mario Scandiffio, is a fourth-year medical student who

gives 21 hours a week service for the sum of eight hundred dollars. He transfers all cultures, keeps the records, and outlines the necessary letters.

"According to records in Washington, sales of fungi from January to December 10, 1927, numbered about 260, and seven cultures were given in exchange to parties who had contributed to the Collection. Sales of fungi were as follows: Aspergilli, 26 per cent; Penicillia, 18 per cent; Mucors, 19 per cent; Phytophthorae and Pythii, 10 per cent; and miscellaneous, 27 per cent. Of the miscellaneous cultures sold, 55 per cent were plant parasites, which means that, including the Phytophthorae and Pythii, 23 per cent of the fungi sold were plant parasites. At least 68 per cent of the sales were from fungous cultures contributed from the Thom and Church collection: namely, Aspergilli, Penicillia, Mucors, and miscellaneous. Therefore, 32 per cent of the sales were of fungi contributed by those not directly caring for the collection.

"According to records in Chicago for the collection as a whole, 3,897 cultures of micro-organisms were sold from a collection numbering 987. Three-hundred and seventy-seven institutions and 115 individuals were furnished cultures.

"The 1927 catalogue of all micro-organisms in the American Type Culture Collection and the cultures may be secured by addressing Dr. George H. Weaver, 637 South Wood Street, Chicago, Ill. To aid in meeting the expense of maintaining the collection, a charge of one dollar per culture, with addition for packing and postage, has been established."

Phytopathological Classics. The editors of *Phytopathological Classics* reported that they had two or three more papers in the series under way. These they hope to issue in 1928. The Business Manager reported that Classic Number 1 cost $232.90 to manufacture. The income from sale of copies to date has been $112.18.

Committee on Permanent Endowment. The committee on an endowment for PHYTOPATHOLOGY reported through its Chairman that it had been unable to come to an agreement as to the need for securing an endowment and so no active steps had been taken in that direction. On motion of J. G. Brown, of Arizona, it was moved that the committee be relieved of further responsibility and that a new committee be organized. An amendment was offered by W. H. Rankin, of New York, to have the committee consist of five members. The motion as amended was passed and the President later appointed E. C. Stakman, Chairman, J. G. Brown, H. H. Whetzel, A. J. Riker, and L. R. Hesler.

Auditing Committee. The auditing committee reported through its Chairman, W. H. Tisdale, as follows:

"We have examined the foregoing accounts of PHYTOPATHOLOGY and the American Phytopathological Society for the year 1927 and find them to be correct."

Elections Committee. The committee on elections reported that ballots had been counted and officers elected as reported in the first portion of this report.

Resolutions Committee. J. C. Walker, Chairman, submitted the following resolutions:

> *Resolved*: That the members of the American Phytopathological Society hereby extend their hearty thanks to Professor Jesse M. Shaver and to the other members of the local arrangement committee for their faithful efforts in aiding the success of the Nashville meeting of the Society.
>
> *Resolved*: That the members of the American Phytopathological Society hereby express their appreciation to the Nashville Chamber of Commerce for their generous help and cooperation during the preparation for and the holding of its 1927 annual meeting.
>
> *Resolved*: That the members of the American Phytopathological Society hereby express their appreciation of the devoted attention and help received

during the Nashville session from Director W. W. Carpenter and Professors W. H. Yarbrough, C. MacMurry, and R. O. Beauchamp, of the Peabody Demonstration School, and their assistants.

Whereas, The American Phytopathological Society recognizes the great service that is being rendered by the U. S. Department of Agriculture in preventing or delaying the entrance of foreign plant parasites into the United States, thus affording invaluable protection to the plant growers of the Nation.

Whereas, The scientific and logical procedure is to supplement this service by investigating, in regions where they are now endemic, all natural and artificial methods of controlling those diseases and pests which have not reached or at least have not become widely established in the United States.

Therefore be it resolved:

1. That the American Phytopathological Society records its appreciation of the work of the U. S. Department of Agriculture in initiating field investigations abroad in cooperation with scientists in foreign countries for the purpose of studying potentially dangerous parasites already introduced, or likely to be introduced.

2. That the Society urge additional support for the extension of this important work, and,

3. That a committee of three be appointed by the incoming President of the American Phytopathological Society to cooperate with a similar committee of the Association of Economic Entomologists, with the Tropical Research Foundation, and with any other interested organizations in furthering this project in whatever manner seems most feasible.

Permanent Committee on Necrology. The committee reported the decease of two members during the year, Erwin F. Smith and P. J. O'Gara. The formal report will be found at the end of record.

Report of Special Committee Appointed to Edit Abstracts. The committee on editing abstracts for the Nashville meeting presented the following recommendation:

"That the closing date for receipt of abstracts be advanced to November 1, and that the committee shall be appointed and notified prior to that date.

"That the Secretary be authorized to return to the author for revision any abstracts which exceed the limit of 200 words, or which the committee considers not to conform to the standards of a meritorious contribution carefully prepared for publication.

"Returned abstracts may be resubmitted not later than November 20, but no paper shall be included in the program until it has been approved by the committee."

Committee on Plant Development. No report.

ACTION OF THE COUNCIL

In addition to making the appointments of the officers mentioned earlier in this report the Council submitted the following actions which were approved by the Society.

1. With regard to the matter of determining a policy with respect to sustaining life members who are delinquent in paying their dues, the Council instructed the Secretary to make such equitable adjustment as he may see fit.

2. The plans that have recently been announced in Science for the International Botanical Congress in London in 1930 were discussed to some extent and it was voted to ask Dr. Donald Reddick, who was special Secretary for our Society for the Ithaca Congress, to negotiate with the British Committee urging an adequate number of special sections for papers and business of strictly phytopathological subjects.

3. It was voted to provide for editorial and clerical assistance for the editors of PHYTOPATHOLOGY in an amount not exceeding $300 for 1928. The University of Minnesota has contributed about $300 per annum up until this year.

4. The recommendations of the special committee on the editing of abstracts were approved and recommended to the Society.

MISCELLANEOUS BUSINESS

At the conclusion of the paper by J. H. Craigie entitled "Heterothallism in the Rust Fungi," the following remarks and motion were made by A. G. Johnson:

"Mr. Chairman: I think we shall all agree that what Mr. Craigie and Dr. Buller have given us in the present paper is of far-reaching importance in many directions. Certainly this is an epoch-making contribution, in many ways ranking along with De-Bary's and Oersted's incontrovertible establishment of heteroecism in the fungi.

"In view of this, Mr. Chairman, I would move that both Mr. Craigie and Dr. Buller be most highly commended for their important contribution, and I would move, further, that the necessary steps be taken to put forward Mr. Craigie's paper for very earnest consideration for the Association's $1,000 prize."

The motion was unanimously carried and the Chairman (Dr. Overholts) after consultation with Professor Barss appointed a committee, composed of A. G. Johnson, E. C. Stakman, and R. J. Haskell, to recommend this paper for the prize. The recommendation to Robert J. Terry, Chairman of the Committee on Award follows:

"We are submitting herewith a paper by J. H. Craigie entitled "Heterothallism in Rust Fungi" which we believe deserves very earnest consideration for the American Association for the Advancement of Science's $1,000 prize.

"This paper was presented Friday forenoon, December 30, before the joint session of the Mycological Section of the Botanical Society of America and the American Phytopathological Society. Following the presentation of the paper, the joint session voted unanimously that the paper be forwarded for very earnest consideration for the American Association for the Advancement of Science prize.

"We consider the paper of far-reaching, epoch-making importance because of the following reasons:

"1. It demonstrates that an organ which heretofore has been considered vestigial and functionless is of primary importance in the developmental cycle of the rusts, one of the most scientifically interesting and practically important groups of parasitic fungi.

"2. It demonstrates heterothallism (the presence of sexual strains) for the first time in the rust fungi.

"3. It furnishes a simpler and more reliable technique than has heretofore been available, for investigations of hybridization between species and parasitic strains of rust fungi, thus offering the possibility of studying experimentally the role of hybridization and consequent recombination and segregation in the origin of species and parasitic strains in this important group of fungi."

The Southern Division voted to make their next meeting a field meeting in some central location in the Southern States.

R. J. HASKELL, *Secretary.*

IN MEMORIAM

ERWIN FRINK SMITH, 1854–1927

Erwin Frink Smith was graduated from the University of Michigan in 1886, and received the degree of Sc. D. from the same institution in 1889.

From 1886 to the date of his sudden death, he was on the staff of the U. S. Department of Agriculture. For twenty-three years he was in charge of the Laboratory of Plant Pathology in the Bureau of Plant Industry.

For his monumental work on the bacterial diseases of plants, and particularly on the relation of some of these diseases to cancer in man and other animals, he received greater recognition than has been accorded to any American plant pathologist. He was sometime President of this Society, of Section G (Botany) of the American Association for the Advancement of Science, of the Society of Plant Morphologists and Physiologists, of the Society of American Bacteriologists, the Botanical Society of America, and of the American Association for Cancer Research. He was a member of the National Academy of Science. He received the honorary degrees of Sc.D. from the University of Wisconsin, and LL.D. from the University of Michigan.

He was a man of broad culture, deeply read in philosophy and in the poetry and literature of several languages. He was himself a poet of no mean achievements.

"Undoubtedly the future holds opportunities equal to those he saw and grasped. Undoubtedly there are others who will accept them, but not many who can do as he did."

PATRICK JOSEPH O'GARA, 1872–1927

Patrick Joseph O'Gara was graduated from the University of Nebraska in 1902, and received the honorary degree of Sc. D. from the same institution in 1917.

From 1902 to 1910 he was a member of the Office of Fruit Disease Investigations of the Bureau of Plant Industry, engaged principally in pear-blight control. From 1910 to 1914 he was plant pathologist and entomologist for the Rogue River Valley, Oregon, engaged principally in the control of pear-blight and frost injury. From 1914 to 1925 he was Director of Agriculture and Smelter By-Product Investigations for the American Smelting and Refining Co. at Salt Lake City.

He was the author of many papers dealing with plant pathology, entomology, agricultural engineering, and mathematics. His brilliant and restless mind ranged over a wide field of interest. He was a skillful mechanic, and expert electrical engineer, and the higher mathematics was his recreation. Essentially an engineer in outlook and methods, his work found expression in control methods now widely practised. Pending the publication of his extensive smoke-injury investigations it is impossible properly to evaluate his work.

REPORT OF THE NINTH ANNUAL MEETING OF THE CANADIAN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The meeting was held at the Dominion Rust Research Laboratory of the Agricultural College at Winnipeg, Manitoba, December 20–21, 1927.

The officers of the society for 1928 are as follows:

President	D. L. BAILEY
Vice-president	F. L. DRAYTON
Secretary-Treasurer	D. R. SANDS
Members of Council	J. DEARNESS and G. R. BISBY

D. R. SANDS, *Sec'y-Treas.*

ABSTRACTS

The reaction of wheat varieties to inoculations with Ophiobolus graminis Sacc.—R. C. RUSSELL.

Seventy-five varieties of wheat were inoculated in the greenhouse with a culture of *Ophiobolus graminis* Sacc., isolated from Saskatchewan field material. The inoculum consisted of a pure culture of the organism growing on moist, ground, oat hulls. Six grams of this was spread in each six-inch pot at seed level when the wheat was being sown. Every variety was tested three times, and a total of approximately 150 seedlings of each variety was exposed to infection. Each test lasted five weeks. At the end of this period the inoculated plants were stunted to about 40 per cent of the height of the checks, and about 60 per cent of them were dead. There were, however, slight but fairly consistent differences in varietal susceptibility noticeable. Good correlation coefficients were obtained between the percentages of dead plants of each variety in the different tests. The resistance shown by the strongest of these varieties was not considered great enough to warrant their use in fields infested with take-all.

Results of experiments on the control of barley stripe.—J. E. HOWITT and R. E. STONE.

Barley stripe, caused by *Helminthosporium gramineum* Rabh., causes very considerable losses in Ontario. The amount of stripe varies considerably in different years. In years when stripe is present, different varieties vary in the amount of stripe present. For the three years 1924–1926–1927 the average amount was as follows: Common six-rowed 16.33 per cent; Bearer (Ottawa No. 475) 6.67 per cent; O. A. C. 21, 3.33 per cent; Winnipeg No. 2, 0.33 per cent.

In our treatments the hooded variety Success was used, as it is susceptible to stripe. The following treatments were used. Semesan ¼ per cent solution soaked 2 hours at 22° C.; Semesan ¼ per cent solution soaked 1 hour at 45° C.; Semesan dust 3 ounces a bushel; Uspulun ¼ per cent solution, 2 hours at 22° C.; ¼ per cent solution, 1 hour at 45° C.; Uspulun dust 3 ounces a bushel; Dupont No. 12 dust 3 ounces a bushel; Bayer's dust 3 ounces a bushel; Copper carbonate 3 ounces a bushel; Vitriolene 3 ounces a

bushel; Formalin sprinkle 1 pint to 30 gallons of water. Dry formalin spray 1–1. The formalin sprinkle prevented germination. All the other treatments reduced the amount of stripe and also increased the yield.

Dupont dust No. 12 and the ¼ per cent solutions of Uspulun and of Semesan applied at 45° C. for an hour gave complete control. The other treatments were less effective.

It is necessary to continue these experiments in order to secure reliable results.

Reaction of Linum species of various chromosome numbers to rust and powdery mildew.—A. W. HENRY.

Linum species representing several groups based on chromosome numbers, for example *Linum grandiflorum* (2× = 16), *L. perenne* (2× = 18), *L. angustifolium* (2× = 30 or 32), and *L. catharticum* (2× = 57?) were used in these studies. Certain strains of *L. usitatissimum*, *L. angustifolium*, *L. crepitans*, *L. rigidum*, and *L. sulcatum* proved completely susceptible; while other strains at least of the first two species, as well as *L. perenne*, *L. austriacum*, *L. grandiflorum*, *L. flavum*, and *L. catharticum*, proved immune when inoculated with *Melampsora lini liniperda* from *L. usitatissimum*. *Melampsora lini cathartici* from *L. catharticum*, a rust supposedly confined to that host, also infected *L. rigidum* normally, but all other species of *Linum* tested were immune. An *Oidium* sp. which developed abundantly on all varieties of *L. usitatissimum* tested in the greenhouse at Cambridge University, England, also produced identical symptoms on *L. angustifolium* and *L. crepitans*. All other species tested proved immune except *L. perenne*, *L. austriacum*, and *L. rigidum*, which developed traces of infection but which are apparently highly resistant. The results of the inoculations with *M. lini liniperda* and *Oidium* sp. support previous evidence from crossing experiments and chromosome counts that *L. angustifolium* is the wild ancestor of *L. usitatissimum*. They also indicate that *L. crepitans* is closely related to these two species.

The haustorium of Cuscuta gronovii.—E. M. MOSS.

Haustorial connections of *Cuscuta gronovii* Willd., with the following hosts were examined: *Monarda mollis* L., *Lathyrus ochroleucus* Hook., *Artemisia gnaphalodes* Nutt., and *Symphoricarpos racemosus* Michx. Uninterrupted union between the xylem strands of host and parasite is made by haustorial tracheids, as described by earlier investigators of *Cuscuta;* but connections between phloem strands of host and parasite by sieve tubes in the haustorium do not seem to occur. Haustorial hyphae, each with a single, hypertrophied nucleus, commonly penetrate the host phloem, where they effect considerable destruction of tissue and ramify in a coralloid fashion, some of the branches entering the host cells and others insinuating themselves amongst the cells. Other hyphae may penetrate series of cells in the cortex or in the pith of the host. The haustorium and stem of the parasite contain starch in abundance, while the host tissues have little, if any, of this carbohydrate. The enlarged penetrating hyphae commonly contain numerous minute starch grains.

Physiologic forms of wheat stem rust in Canada.—M. NEWTON, T. JOHNSON, and A. M. BROWN.

By greenhouse experiments, 24 physiologic forms of *Puccinia graminis tritici* have been shown to be present in Canada. Five of these forms are different from those reported by Stakman and his co-workers in the United States. Some evidence has been found suggesting a possible relationship between the varieties of wheat grown and the distribution of physiologic forms of rust.

In the course of determining the physiologic forms in 1927, thirty-three separate cultures of aeciospores from barberries, all but one of which had been artificially inoculated, were transferred to wheat plants in the greenhouse. In 14 of these wheat cultures, some greyish-brown pustules appeared. These were obtained in pure culture, and, as in the earlier case reported in Phytopath. 17: 711–725, 1927, they have remained constant in color, producing only greyish-brown uredinia. For most of these barberry infections, telia from *Hordeum jubatum* were used as a source of inoculum. (Dominion Rust Research Laboratory.)

Treatment of millet seed to prevent smut.—R. E. STONE.

Millet smut is a common disease of foxtail millets in Ontario, caused by the fungus *Ustilago cramerei* Koern.

Seed from a badly smutted crop was treated in several ways. The untreated seed gave a crop 56 per cent smutted. Formalin sprinkle and formalin soak prevented germination of seed. Dry formaldehyde treatment markedly reduced the amount of smut but also checked germination to some extent. Bluestone soak and Bayer's dust were not effective. Semesan ¼ per cent solution and Uspulun ¼ per cent solution were quite effective, reducing the amount of smut to 1.9 per cent and 0.9 per cent, respectively. Dupont No. 12 dust reduced the amount of smut to 0.6 per cent, while copper carbonate reduced the amount to 1 per cent.

These experiments will be continued.

Sexual behavior of Puccinia graminis.—J. H. CRAIGIE.

An experimental investigation upon sex in the stem-rust fungus has demonstrated that it is heterothallic. The sporidia are of two kinds (+) and (−). A (+) sporidium gives rise to a (+) mycelium and a set of pycnia which produce (+) pycnospores. A (−) sporidum gives rise to a (−) mycelium and a set of pycnia which produce (−) pycnospores. When a (+) sporidium and a (−) sporidium are sown close together on a leaf, the (+) and (−) mycelia resulting therefrom intermingle and produce diploid aecia. When (+) pycnospores are brought into contact with (−) pycnia, or (−) pycnospores with (+) pycnia, diploid aecia are produced on the under side of the pustule receiving the pycnospores within a few days of the transference. There is therefore the possibility of two different strains of this rust crossing and producing a new physiologic strain of rust. The experimental results will be found in "Nature," Vol. 120, No. 3012 and No. 3030, 1927. (Dominion Rust Research Laboratory.)

Physiologic forms of Puccinia graminis avenae Erikss. & Henn., in Canada.—W. L. GORDON.

In 1924, Bailey reported the occurrence of five physiologic forms of *Puccinia graminis avenae*. Forms 1, 2, and 5 were found to be present in Canada.

More extensive collections of oat stem rust were made during 1925–1927. Forms 2 and 5 have predominated each year. Form 1 has been isolated infrequently.

A study of the heterogeneous or X reaction, given by form 5 on two differential hosts, was made by the single-spore method. The heterogeneous reaction occurred again, when inoculations were made with single-spore cultures.

Physiologic forms more virulent than 1, 2, and 5, first collected in 1925, have appeared each year. Form 3 has been collected once, but form 4 has appeared more frequently. A collection from Paskwegin, Sask., in 1925, yielded a form which differs in its infection capabilities from all other forms yet reported in being able to infect

heavily all differential hosts. It is considered new, and has been named physiologic form 6.

Seedling tests for rust resistance, of some 230 varieties or strains of oats from various sources in America, as well as more than 100 varieties from France, Germany, Sweden, and Russia, have been carried out in the greenhouse to physiologic forms 4 and 6. All were quite susceptible to form 6, and only one variety, from France, gave any indication of resistance to form 4. In a field test, these forms appeared to be equally virulent. (Dominion Rust Research Laboratory.)

A seedling blight disease of oats caused by Fusarium culmorum.—P. M. SIMMONDS.

The symptoms were described of the seedling blight of oats caused by *Fusarium culmorum*. Some observations on pentration and invasion of the oat seedling were reported. Penetration may take place through the mesocotyl or coleoptile. The fungus particularly invades the cortical tissues. Mycelium may collect between the coleoptile and plumule. At the crown, tiller buds may be invaded as well as root primordia. Some evidence has been obtained that entrance may take place through root hairs.

Sulphur dusting for the control of leaf and stem rust in Manitoba: Winnipeg small-plot experiments.—F. J. GREANEY and D. L. BAILEY.

A series of one-fortieth acre plots was dusted with Kolo-dust at Winnipeg to determine the influence of the rate, frequency, and time of application of the dust on resulting rust control. A very heavy epidemic of leaf and stem rust developed during the season. The effectiveness of the method was conclusively demonstrated and the degree of control was in general proportional to the rate and frequency of the applications. Tri-weekly applications at 45 pounds an acre reduced the infection percentage of stem rust as compared with the check from 87 to 4 per cent and increased the yield from 12 to 48 bushels an acre. The only fortnightly application which was significantly effective was the 45-pounds-an-acre one and this did not compare favorably with weekly applications at lighter rates. From the practical standpoint it appears that bi-weekly applications at the rate of 30 pounds an acre or weekly ones at 45 pounds are most desirable for a bad rust year. The final choice will depend on the cost of application. Results indicate the desirability of beginning dusting as soon as traces of rust appear and continuing until the crop is practically mature. (Dominion Rust Research Laboratory.)

Sulphur dusting for the control of leaf and stem rust in Manitoba: Field trials with horse-drawn and aeroplane dusters.—D. L. BAILEY and F. J. GREANEY.

A horse-drawn duster applied 30 pounds of Kolo-dust an acre once a week for eight weeks on acre plots at Winnipeg, and the treatment reduced the infection percentage of stem rust as compared with the check from 90 to 40 per cent and increased the yield 20 bushels an acre. This result was as good as those obtained with bi-weekly applications at 15 pounds an acre. The net increase in value over the checks was $15.28 an acre.

Aeroplane dusting was carried on in three localities. The results were splendid in one case, mediocre in a second, and negative in the third. The negative results indicated that in a bad rust year like 1927 dusting should be begun when only a trace of rust has developed, that 30 to 45 pounds an acre a week should be used and that dusting should be continued until the crop begins to mature. The positive results increased the value of the crop in one place by $40.00 an acre. The aeroplane was thoroughly suited to this type of work especially on large continuous acreages. (Dominion Rust Research Laboratory.)

The dwarf leaf rust of barley in Western Canada (Puccinia anomala Rostr.).—A. M. BROWN and M. NEWTON.

This rust was first collected in Western Canada in 1922, but was not again found until this year when it was quite abundant in southwestern Manitoba and also at Indian Head, Saskatchewan.

Urediniospores were germinated in hanging drops of distilled water at different temperatures. The optimum for spore germination appears to be from 11° C. to 17° C.

A large percentage of urediniospores kept in the laboratory at room temperature, germinated at the end of a month, but at the end of three months no germination was observed. (Dominion Rust Research Laboratory.)

Cereal diseases in Alberta in 1927.—G. B. SANFORD.

The following are the chief results of the first extensive plant disease survey in Alberta. More than a trace of stem rust on wheat rarely occurs in Alberta. However, under the extremely favorable conditions of abundant moisture and a prolonged ripening period, an unusual amount of this rust developed over the entire area, from the northern limits of the survey (Athabasca) to the Montana boundary. Actual shrinkage occurred in the Camrose area, but very little injury elsewhere. Slight infection of stem rust of oats developed as far south as Lethbridge and north to Edmonton. Leaf rust of wheat was very prevalent and in many cases severe. Stripe rust (*Puccinia glumarum*) was found again at Olds on the leaves and glumes of wheat, and on nearby Hordeum jubatum; also in wheat fields and on *H. jubatum* in the southernmost part of the province. Glume blotch of wheat (*Septoria glumarum*) was very prevalent and severe. Slight infections of basal glume rot (*Bacterium atrofaciens*) and also of what appears to be black chaff of wheat (*B. translucens*) were collected at diverse points. Root rots of wheat were severe and are the most important plant diseases of Alberta, causing a loss estimated at 7,000,000 bushels. A distinct correlation of the severity of root rots with the black soil type was noted.

The Occurrence of yellow stripe rust in western Canada.—T. JOHNSON and M. NEWTON.

Stripe rust (*Puccinia glumarum*) was discovered at Edmonton, Alberta, in 1918, by Professor W. P. Fraser. Since then it has occurred annually on *Hordeum jubatum* in certain localities in Alberta. On barley it has been found once only. On wheat it was first observed in 1926 at Olds, Alberta, and again in 1927. Other hosts found naturally infected are *Agropyron smithii* and *Agropyron tenerum*. The area in which stripe rust has been found now includes scattered localities from Edmonton to the international boundary as well as southwestern Saskatchewan.

Attempts were made to determine whether the rusts on *H. jubatum* and on wheat were the same form or two distinct, specialized forms. Difficulty was experienced in establishing greenhouse cultures of the rust, probably on account of poor spore germination. Finally two cultures were established, one from wheat, the other from *H. jubatum*. Both of these proved to belong to the *P. glumarum tritici* form. Although this does not prove that only that form exists in Canada, it shows that the rust on *H. jubatum* may under favorable conditions affect wheat.

Owing to difficulties in obtaining artificial infection, an attempt was made to determine optimum conditions for germination of fresh urediniospores produced in the greenhouse. Various methods were used at 10° C., 15° C., 17° C., 19° C., and 23° C., but under all conditions tried germination remained low and irregular. Germination was best at 10° C. and 15° C., the average being only about 8 per cent in each case. (Dominion Rust Research Laboratory.)

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